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PAPER CHROMATOGRAPHIC DETERMINATIONS OF AMINO ACIDS IN THE SALIVARY GLANDS OF CERTAIN DIPTERA

JOHANNA BLUMEL*

The methods of partition paper chromatography introduced by Consden, Gordon, and Martin (1944) are providing a useful tool for the separation of amino acid mixtures in very small amounts of material. This method is also useful in the qualitative and quantitative study of the composition of nucleoproteins, their constituents and derivatives, and in the manner of their hydrolytic degradation.

In the present investigation, an attempt is being made to utilize this technique to determine the amino acid composition of the giant salivary glands of certain Diptera. These cytological objects are ideal for these analyses.

Materials and Methods

The stocks used in this investigation are given below:

Drosophila virilis Sturtevant. This is a lethal-free and fertile strain from Pasadena. In this paper it will be referred to as *virilis*.

Drosophila americana texana, New Orleans strain 1128.10. This stock is also lethal free and fertile, and will be referred to as *texana*.

Drosophila repleta, Mexico City strain 1797.1. We will refer to this stock as *repleta*.

Drosophila melanogaster. This is the standard Oregon stock and will be referred to as *melanogaster*.

Megascelia sp. This stock was isolated and cultured at the Texas laboratory. This is a genus belonging to the Dipterous family *Phoridae* and will be referred to as the *Phorid*.

For analysis salivary glands were isolated from larvae of *virilis*, *texana*, *repleta*, *melanogaster*, and *Phorid*. Two hundred pairs of glands were used for each test. All of the glands were isolated from healthy, well-fed, mature larvae which had been raised in half-pint milk bottles in a constant temperature room 22° to 24° C. The standard medium of banana-malt-extract-yeast agar of the Texas laboratory was employed. To insure an adequate food supply for maximum growth, avoid overcrowding, and give uniform material

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for dissection; the developing larvae were spread to fresh food bottles two or three times during their larvae stage.

Separation of the amino acids was effected by the Williams and Kirby (1946) paper chromatography technique. The salivary glands were hydrolysed in 3N hydrochloric acid for 35 hours at 100° C. After hydrolysis the hydrochloric acid was removed by evaporation at a low temperature under reduced pressure and the residue was taken up in 0.200 ml. of water. An aliquot of this, 0.050 to 0.100 ml., was used to prepare two-dimensional chromatograms, using phenol as the first solvent and collidine as the second.

Results

The amino acid composition of the salivary glands investigated is summarized in Table 1. The quantitative differences in the salivary glands analyzed are presented in Table 2. By comparing the size of the spots and by successively diluting the hydrolysates quantitative data were obtained. On all of these determinations at least three separate tests were made.

Since some amino acids are destroyed during acid hydrolysis, several attempts were made to produce alkaline hydrolysis.

TABLE 1

Qualitative Amino Acid Composition of Salivary Glands

	Salivary Glands				
Amino Acids	**Virilis**	**Texana**	**Repleta**	**Melanogaster**	**Phorid**
Aspartic	+	+	+	+	+
Glutamic	+	+	+	+	+
Serine	+	+	+	+	+
Glycine	+	+	+	+	+
Lysine	+	+	+	+	+
Threonine	+	+	+	+	+
Alanine	+	+	+	+	+
Arginine	+	+	+	+	+
Valine	+	+	+	+	+
Leucine	+	+	+	+	+
Tyrosine	+	+	+	+	+
Phenylalanine	+	+	+	+	+
Proline	+	+	+	+	+
γ-aminobutyric	—	—	—	—	+
Taurine	+	—	+	+	+
Ethanolamine	+	+	+	—	+
Cystine	+	—	—	—	—
X_4	+	—	—	—	—

TABLE 2

Quantitative Amino Acid Data

Amino Acids	Micromoles per 100 salivary glands			
	Virilis	Texana	Repleta	Melanogaster
Aspartic	0.376	0.376	0.376	0.188
Glutamic	0.680	0.680	0.680	0.340
Serine	0.238	0.476	0.238	0.114
Glycine	0.333	0.160	0.666	0.333
Lysine	0.342	0.342	0.342	0.171
Threonine	0.840	0.840	0.840	0.420
Alanine	0.280	0.280	0.280	0.280
Arginine	0.144	0.086	0.144	0.144
Valine	0.854	0.427	0.854	0.427
Leucine	0.763	0.382	0.763	0.382
Tyrosine	0.166	0.331	0.221	0.039
Phenylalanine	0.152	0.242	0.303	0.303
Proline	0.522	0.348	0.435	0.522

These and other efforts to determine any free amino acids that might be present were unsuccessful.

Figure 1 is a tracing of a chromatogram showing the amino acids present in the different hydrolysates. Spots reacting to give a typical ninhydrin reaction and now identified as gamma aminobutyric acid, taurine, ethanolamine and cystine were previously reported as X_1, X_2, X_3, and X_5 respectively for *virilis* tissues by Blumel and Kirby (1948). They also reported spot X_4 and as yet no spots identical to it have been reported; however, differences in conditions during the preparation of the chromatograms are such that it is difficult to compare them with those made by other workers.

In order to obtain satisfactory and consistent results, at least five thousand salivary glands were isolated for amino acid analysis.

Discussion

The method of two-dimensional ascending partition paper chromatography indicates that an analysis of this type will be helpful in elucidating the chemical composition and ultimately the structure of the chromosomes. In this search for more knowledge of the organization and exact chemical composition of

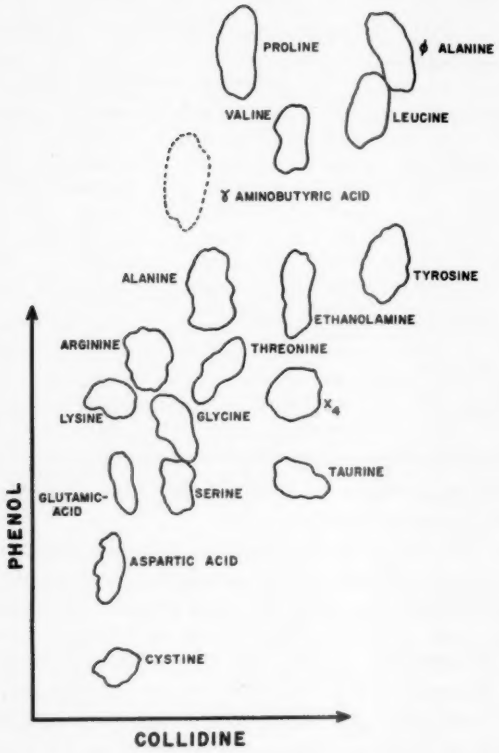

FIG. I. Tracing of a Two-Dimensional Paper Chromatogram of 36 Hour Hydrochloric Acid Digest of Giant Salivary Gland Chromosomes of Virilis. X_4 is as yet an unidentified substance. Gamma-aminobutyric acid is present in *Phorid* and is shown here to give its position on the chromatogram.

chromosomes in nuclear cytology and chemistry it must be kept in mind that one is dealing with the products of hydrolysis. This may lead to incomplete cleavage products or to the destruction of certain compounds and thus make detection impossible. An example of the latter would be the destruction of tryptophane and cystine by acid hydrolysis. Conversely, hydrolysis may lead to the production of artefacts. These difficulties, of course, are encountered in any analysis of a complex organic substance.

In this investigation salivary glands from four species of Dro-

sophila and one species of non-Drosophila were analyzed for their amino acid content.

An inspection of Tables 1 and 2 will show certain differences in the amino acid components of even very closely related species such as *virilis* and *texana*. The differences in the substances reacting to give the ninhydrin reaction is greater in these two species than in the less closely related ones, such as *repleta* and *virilis*.

On a qualitative basis there is no difference in the so-called naturally occurring amino acids in the glands analyzed from the several different forms. There is, however, a difference in some of the compounds usually considered as not occurring naturally —a difference which seems to be greater in *virilis* and *texana* particularly when one considers the close relationship of these two species. Only ethanolamine is present in *texana;* whereas, in *virilis* there is taurine, ethanolamine, cystine and X_4. *D. repleta* contains taurine and ethanolamine, in addition to the thirteen amino acids that are common to all the glands analyzed. The *Phorid* contains gamma-aminobutyric acid; this amino acid is not present in the salivary glands of the four species of Drosophila analyzed. The other two compounds, in addition to the thirteen amino acids present in all of the glands analyzed, were taurine and ethanolamine. Actually *Phorid* has some of the compounds common to all of the salivary glands analyzed: one each of *melanogaster* and *texana* and two of *virilis* and *repleta*.

Quantitatively there is a greater discrepancy between *virilis* and *texana* than between *virilis* and *repleta*. Aspartic acid, glutamic acid, lysine, threonine, and alanine are present in approximately the same concentration in *virilis*, *texana* and *repleta*. In addition to these, *repleta* and *virilis* also have serine, valine, and leucine in the same concentration. While nine of the thirteen acids are present in the same concentration in *virilis* and *repleta*, only five of the thirteen are present in the same concentration in *virilis* and *texana*.

The salivary glands of *virilis*, *texana* and *repleta* are about the same size; however, only the weight of the *virilis* glands was determined; therefore, the comparison of the relative amounts of amino acids present are in relation to each other. The glands of *melanogaster* are about one-half the size of the other three species and it is, therefore, difficult to compare them. It is worthy of

note that in several instances the concentration of a particular amino acid in *melanogaster* is comparable to the highest concentration encountered in the other three species; that is, proline is present in equal amounts in *virilis* and *melanogaster*. Glycine and arginine are also present in the same concentration in *virilis* and *melanogaster*. Alanine is the one amino acid that is present in all four species in the same concentration. It is probably well to point out that this particular amino acid was found also in isolated chromosomes of *virilis* (Blumel and Kirby, 1948). In *texana* and *melanogaster*, in addition to alanine, leucine and valine are also present in the same concentrations. Phenylalanine, arginine and alanine have the same concentration in *repleta* and *melanogaster*. In this respect *virilis* and *melanogaster* seem to be somewhat more alike.

The values for the glands of *virilis*, *texana*, *repleta* and *melanogaster* were given in micromoles in Table 2. The values in micrograms are as follows: *repleta* 765, *virilis* 715, *texana* 642, and *melanogaster* 454. It is difficult to draw any conclusions from these results. As stated previously the glands of *virilis*, *repleta*, and *texana* are approximately the same size. Although *repleta* apparently has a higher amino acid content, examination of Table 1 shows that in addition to the thirteen naturally occurring amino acids only taurine and ethanolamine are present; whereas *virilis* has taurine, ethanolamine, X_4 and cystine. It is entirely possible therefore that these two species have approximately the same total amino acid content. Because of the smaller size of the *melanogaster* glands, 454 micrograms is a reasonable amount for this species. A greater discrepancy exists between *virilis* and *texana*. Since in *texana* ethanolamine is the only other compound that gives the ninhydrin reaction in addition to the thirteen naturally occurring amino acids, the total amount of amino acid present would not be appreciably different. Thus, as far as the total amino acid content is concerned, *virilis* and *repleta* are more alike, qualitatively as well as quantitatively, than are the more closely related species of *virilis* and *texana*.

Morphologically, *virilis* and *texana* are quite different and it is possible that the difference in the amino acid content of these two species has a definite bearing on this morphological dissimilarity. Perhaps the actual basic hereditary substances which act

as transmission genes are not a host of materials, but a few fundamental protein constituents that control the synthesis of the necessary cell constituents.

Summary

In the experiments described, salivary glands of four species of Drosophila and one member of the Phoridae were analyzed for their amino acid content.

By means of the two-dimensional partition paper chromatography technique, the following amino acids were demonstrated in the hydrolysates of intact salivary glands of *virilis*, *repleta*, *texana*, *melanogaster*, and *Phorid:* proline, leucine, valine, alanine, glycine, glutamic acid, aspartic acid, arginine, lysine, serine, threonine, tyrosine, and phenylalanine. In addition to these, gamma-aminobutyric acid, possibly taurine, and ethanolamine were demonstrated in *Phorid* salivary gland hydrolysate. Taurine was also present in *melanogaster*, *repleta* and *virilis*. Ethanolamine was found to be present in *repleta*, *texana*, and *virilis*. Two other compounds were present in *virilis*, X_4, a completely unidentified compound which gave the ninhydrin reaction, and cystine or a derivative of cystine.

Quantitative differences in the amino acids were demonstrated for the four species of Drosophila tested.

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A FUNCTIONING POLYCYSTIC OVARY SYNDROME IN THE CANCER OF THE BREAST: ITS ROLE IN THE GENESIS OF HYPERESTRINISM

D. BRACHETTO-BRIAN*

A functional and anatomical alteration of the ovary, which we interpret as the principal cause of hyperestrinism, is frequently observed in cancer of the breast. The present studies were carried out in the "Instituto de Medicina Experimental" (1948 to 1952), some of them with the collaboration of Dr. Moguilevsky and Dr. L. K. Srulijes, as we state in the corresponding references.

Temporary and sometimes even surprising improvement, in cases of cancer of the breast after ovariectomy, was observed by Schinzinger[16] as early as 1889 and by Beatson[2] in 1896. This fact was confirmed by all investigators who have studied this problem in a sufficient number of well-controlled patients. More recently, with the same procedure, Taylor[18] obtained temporary regression and remarkable improvement in a third of the cases of recurrent or inoperable cancer of the breast, observing a checking action on bone metastasis also. Experimentation reveals several facts which corroborate these observations. We shall mention two of the most significant discoveries: a) Lathrop and Loeb,[9] in mice of a strain with a high incidence of spontaneous mammary cancer (sixty to seventy per cent) ovariectomized before six months of age, found that the rate decreased to nine per cent. b) Lacassagne[8] induced breast cancer in male mice of a strain with high incidence of this condition in females (seventy-two per cent), by weekly subcutaneous injections of 0.03 mg. of estrone benzoate dissolved in sesame oil, starting at ten to eighteen days of age. Mammary canceration in the males appeared three and one-half to six months later.

We undertook these series of investigations in order to study the following problems: 1) the mechanism of the drop of hyperestrinism after ovariectomy; 2) the cause of the temporary improvement obtained by ovariectomy in some women with advanced cancer of the breast accompanied with hyperestrinism;

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3) the role of the ovary in the genesis of hyperestrinism in cancer of the breast.

Clinical Findings

The clinical findings observed in our series of patients are: 1) hyperestrinism occurs in women with cancer of the breast, independent of their clinical state, in the following proportion: ninety per cent of the thirty to forty-year old patients, fifty per cent of the fifty to fifty-five-year old patients and twenty per cent of the fifty-five to sixty-five-year old patients. These figures show that in cancer of the breast, hyperestrinism is present in a high percentage of young women and in one fifth of the cases of menopausal women. The data are based on the observation of five hundred patients[3,6] and the hyperestrinism was determined by the cytological curve of cornified cells (Fig. 1) ac-

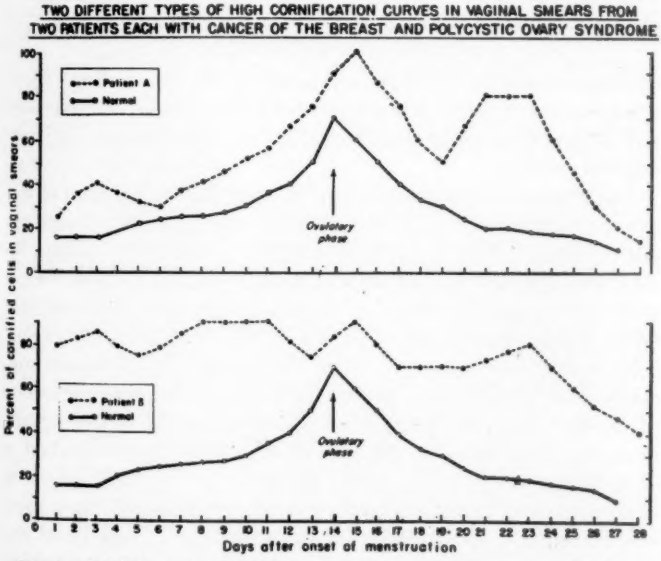
TWO DIFFERENT TYPES OF HIGH CORNIFICATION CURVES IN VAGINAL SMEARS FROM TWO PATIENTS EACH WITH CANCER OF THE BREAST AND POLYCYSTIC OVARY SYNDROME
Patient A
Normal
Ovulatory phase
Patient B
Normal
Ovulatory phase
Percent of cornified cells in vaginal smears
Days after onset of menstruation

FIG. 1. *Ovulatory type.* Patient A: Thirty-six-year-old woman with hyperestrinism during ovulatory, luteal and premenstrual phases as determined by the percentage of cornified cells present in the vaginal smears.
Anovulatory type. Patient B: Twenty-nine-year-old woman with hyperestrinism during all the mentrual cycle as determined by the percentage of cornified cells present in the vaginal smears.
Both patients had premenstrual mastopathy.
Both curves are compared with the cornification curve of a normal woman.

cording to de Allende and Orias.[1] 2) Bilateral ovariectomy[3] produces a drop of the hyperestrinism for three to twelve months, and also more or less marked but temporary improvement in thirty per cent of the advanced cases. This improvement is manifested by a cessation of the progressive growth of the tumor, regression of the bone metastasis and a general state of well-being. In the erysipeloid type, the edema is reduced and the pain is relieved. This improvement may be made more pronounced and lasting by the action of androgen therapy, applied when the curve of cornified cells rises again above the normal. This phenomenon occurs three to twelve months after ovariectomy. 3) We have observed[4] the fact, pointed out by Olch,[10] that fifty per cent of the women with cancer of the breast have a late menopause, so, since the menarche occurs in both about the same age, their sexual lives are longer than those of normal women. This estimate is based on the observation of a hundred cases.

Pathological Findings

The pathological findings are based on the study of two hundred ovaries removed from patients having cancer of the breast and hyperestrinism as determined by the cornification curve in vaginal smears.

The Ovary in Premenopausal Patients. Eighty per cent of the ovaries examined were from thirty to forty-six-year old patients with different clinical types of mammary cancer including the slow growing, the acute type and those with and without lymph node and bone metastasis. The majority of the cases (seventy per cent) had ovulatory cycles and the rest had anovulatory cycles. *The macroscopic examination* of the specimens before and specially after the section, revealed the almost constant presence of two to four cysts. They were generally located in the periphery, measured five to fifteen millimeters in diameter, had a smooth continuous wall and contained 0.25 to 2.80 ml. of a transparent or yellowish fluid. One or two corpora lutea were also found. No hypertrophy of the tunica albuginea or of the connective tissue of the stroma was observed. The section of some ovaries showed an edematous aspect. The weight of each ovary varied from two to seven grams. *The microscopic examination* (serial longitudinal sections, were stained with hematoxylin-eosin and Masson's trichrome. Frozen sections stained with Sudan III), revealed one or two microcysts besides those

visible by gross examination, that is a total of two to six cysts (Fig. 2). The cyst walls were formed (inside to outside) by a granulosa layer (Fig. 3) of four to eight rows of cells in whose

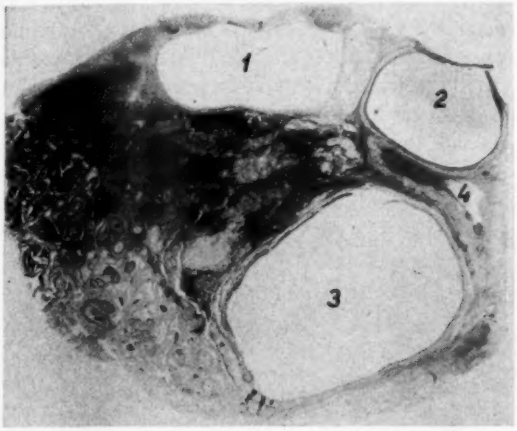

FIG. 2. Topographical section of the ovary (low magnification). Three follicular cysts (1, 2, 3) and corpus luteum (4). The stroma and the tunica albuginea remain normal. The specimen weighed 6.7 gms. and was obtained from a 42-year-old woman in the premenopause and with cancer of the left breast and bone metastasis.

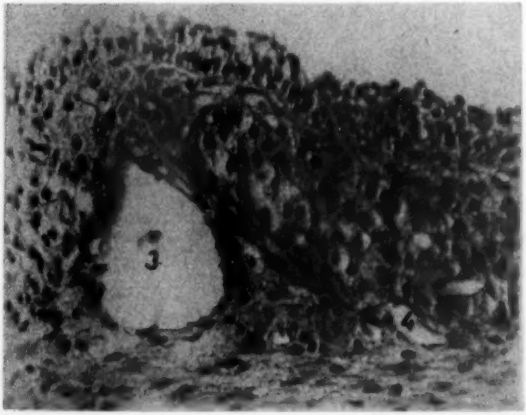

FIG. 3. Detail of the wall of a follicular cyst (high magnification). The granulosa layer (1) and the theca interna (2) are well developed, in lutein evolution. The basement membrane is between both layers. There are large blood capillaries (3, 4) in the theca interna. The specimen was obtained from a 42-year-old woman in the premenopause and with acute cancer of the left breast.

middle region mitoses were frequently found; this layer lay on a basement membrane and circumscribed a cavity partially filled with a homogeneous and coagulated substance. Outside the basement membrane there was a theca interna layer with the following characteristics: a) frequently a manifest proliferation with mitosis, sometimes forming growths which penetrated the theca externa; b) the cellular morphology presented a clear luteinization (Fig. 3) or well defined borders and acidophil cytoplasm, Leydig-like cell type (Fig. 4); in other cysts both types of cells

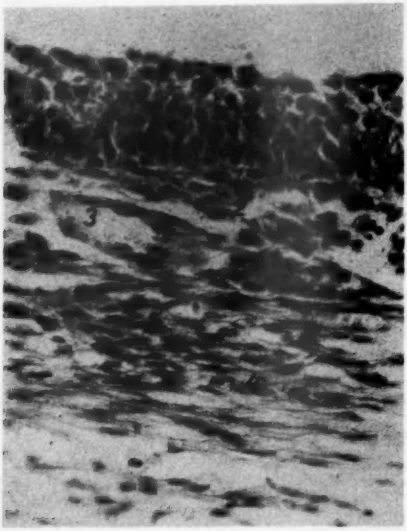

FIG. 4. Detail of the wall of a follicular cyst (high magnification). Well developed granulosa layer (1). Leydig-like type cells of the theca interna (2). Blood capillaries (3). Theca externa (4). The specimen was obtained from a 44-year-old woman in the premenopause and with cancer of the right breast.

were mixed; c) the cells of the theca interna were arranged around numerous dilated blood capillaries, presenting on the whole the architecture of an endrocrine gland.

The structure of some of these follicular cysts revealed regressive processes: disappearance of the intercellular spaces and decrease in the number of the granulosa layer cells, now forming only two to three rows (Fig. 5); in some of the cysts these cells had disappeared by total desquamation and the basement mem-

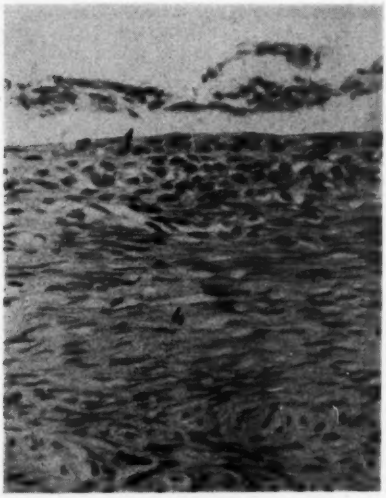

FIG. 5. Detail of the wall of a follicular cyst in regression stage (high magnification). The cells of the granulosa (1) are arranged in two or three rows and reduced in size. Hypertrophy of the basement membrane (2). The cells of the theca interna (3) are also reduced in number as well as in size, and are compactly arranged; the blood capillaries of this layer are scarce. The theca externa (4) in fibro-collagen transformation. The same patient as in Fig. 2.

brane presented hyalinization and hypertrophy; and decreased size and number of the theca interna cells that gather around the blood capillaries. These regressive alterations were less frequent in the theca interna layer than in the granulosa. No process of obliteration like the one undergone by atretic follicles was noticeable.

In some cases we discovered a curious follicular anomaly consisting of primary follicles with a dead ovum, which were situated in the interna or the externa theca layers of a follicular cyst (Fig. 6).

There were always found one to three menstrual corpora lutea (Fig. 1) in various phases of evolution. In some ovaries there were foci of luteinization in the stromal cells. There were also numerous corpora albicantia (ten to fifteen) and corpora fibrosa in each section. There were a fair number of maturing follicles, some with dead ova, Graafian and atretic follicles. Very often we also found microcysts derived from the germinal epithelium

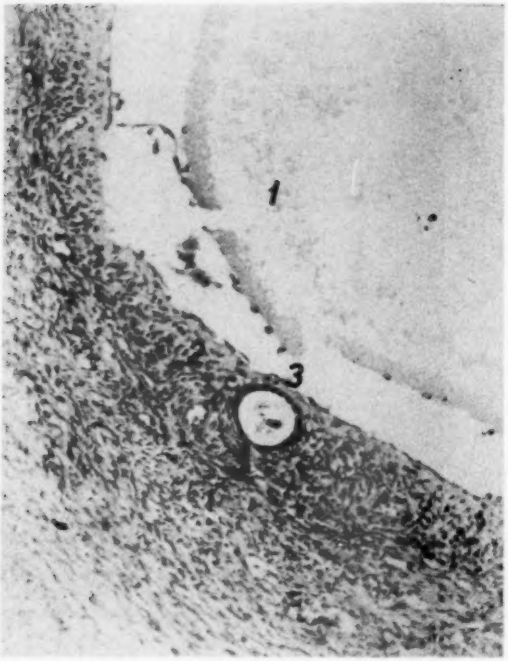

FIG. 6. Malformation of follicular cyst in regression stage (1) (medium magnification). The theca interna layer (2) has a primary follicle (3) at the starting stage of maturation with a dead ovum. The specimen was obtained from a 38-year-old woman with alveolar cancer of the left breast and metastasis in the supraclavicular lymph nodes.

and Wolffian vestiges. In one of the ovaries there was a thecoma.

The Ovary in the Menopausal Patients. As we have pointed out, twenty per cent of the patients of 55–65 years of age had high curves of vaginal cornified cells. Menstruation had ceased spontaneously in one to five years. The ovaries weighed from one to three and one-half grams. Sometimes there were one to two follicular cysts (some in regression) with pronounced hyperplasia of the theca interna which revealed a marked functional activity. The histological picture was completed by the presence of corpora albicantia appearing in such a number in some of the ovaries as to suggest an intense sexual activity during the premenopausal period, also a fair number of atretic follicles and of corpora fibrosa. It was not a rare occurrence to find numerous

microcysts developing out of the germinal epithelium. No primary follicles were found. No hypertrophy either of the tunica albuginea or of the stroma was observed.

Determination of the Estrogenic Action of the Follicular Cyst Fluid

The determination of the estrogenic action of the follicular cyst fluid was done in the cysts of the ovaries removed from nineteen patients. All of them had high cornification curves of vaginal cytology, a test that established the indication for ovariectomy. Sixteen of these patients had inter- or premenstrual mastopathy. Fourteen had ovulatory menstrual cycles and their age range was from twenty-eight to forty-nine years; four had anovulatory cycle and their ages ranged from twenty-nine to thirty-four years, and one fifty-nine-year old patient had an anovulatory menopausal cycle.[5]

The test employed was that of Allen-Doisy, applying the technique of Dr. S. K. de Srulijes[5,17]: after establishing the character of the sexual cycle in adult white mice of approximately twenty grams, bilateral ovariectomy was performed and forty-eight hours afterwards the control by vaginal cytology was started and continued during eighty days; fifteen days after the ovariectomy and twenty-four hours before injecting the fluid, the mice were sensitized with 0.02γ of estrone. By means of transillumination the location of accessible cysts in the specimens was determined, in order to remove the fluid by puncture. It was then injected in decreasing quantities into the sensitized mice. Histological control of the punctured cysts was performed. To establish the gradation of the estrogenic action of the fluid we kept in mind the minimum dose capable of producing cornified cells, in the following progression: $+$, cornified cells and neutrophil leukocytes; $++$, cornified cells and other epithelial cells; $+++$, cornified cells only. We found estrogenic action (equivalent to 0.05γ to 30γ of estrone) in the cysts of the ovaries of fourteen patients, but no estrogenic action in the cysts of the ovaries of five patients. In 73.6 per cent of the cysts the fluid had estrogenic action. Nevertheless, we must consider the possibility of a higher percentage of cysts with estrogenic action having in mind the factors determining negative reactions: namely, when in

three cases removal of fluid was impossible because the cysts were too small, and one, in which the test animal died.

Discussion

I. In Chart I we summarize the principal characteristics of the two most frequent syndromes of functioning polycystic ovaries: *metropathia hemorrhagica* and *sclerocystic ovary*, together with the principal characteristics of the *polycystic ovary syndrome in mammary cancer*. The most remarkable charac-

CHART I

Condition	Metropathia hemorrhagica*	Sclero-cystic ovary	Polycystic ovary in mammary cancer
Menstrual cycle.....	monophasic metrorrhages	monophasic dysmenorrhea	*biphasic*†
Premenstrual mastopathy.....	non-occurring	non-occurring	*frequent*, 84%
Sterility.....	always	frequent	*rare*
Ovary follicular cysts: amount.....	solitary or multiple	multiple in both ovaries	multiple in both ovaries
granulosa layer....	existent	existent	well developed
theca interna layer.	moderate	moderate	*marked hyperplasia*
Corpus luteum.....	non-existent	non-existent	*existent*
Tunica albuginea and stroma of the ovary.	normal	hyperplasia	*normal*
Curve of cornified vagina cells.....	moderate	moderate	*high*
Endometrial cycle: proliferative phase..	occurring	occurring	occurring
secretory phase....	non-occurring	non-occurring	*occurring*

* We refer to the metropathia hemorrhagica caused by functioning follicular cysts of the ovary, also called "Brennecke-Schroeder disease."
† The most important characteristics of the polycystic ovary in mammary cancer are underlined.

teristics of the *metropathia hemorrhagica* and *sclero-cystic ovary syndromes* are: Monophasic menstrual cycle (generally with metrorrhage in the metropathia hemorrhagica and dysmenorrhea in the sclero-cystic ovary); sterility; almost constant lack of corpus luteum; hyperplasia and hypertrophy of the stroma and of the albuginea in the sclero-cystic ovary; the endometrium shows only a proliferative phase in both syndromes.

The most remarkable characteristics that we found in the *poly-cystic ovary in cancer of the breast with hyperestrinism* were the following: biphasic menstrual cycle; frequent premenstrual mastopathy; sterility is rare. The cysts were always smaller than those found in the sclero-cystic ovary and, in general, the ovaries were not palpable; the granulosa layer was well developed and in luteinization; the cells of the theca interna showed marked proliferation, in luteinization of Leydig-like type, round numerous dilated capillaries; mitosis was frequent in both layers: morphologic characteristics that revealed intense functional activity, even in regressive cysts. The fluid had estrogenic action. The finding of corpora lutea in diverse states of evolution was a noteworthy feature. The tunica albuginea and the stroma did not show any alterations. The curve of the vaginal cytology revealed a high and constant percentage of cornified cells. In the majority of the cases we observed the proliferative and secretory phases of the endometrium. As the anatomo-clinical features described in these three syndromes depend in great measure upon functional conditions, they are subject to possible transitory oscillations and variations.

II. The interpretation of the functional pathology of the polycystic ovary in cancer of the breast with hyperestrinism presents an interesting question. In conjunction with the normal evolution of the primary follicles (some after ovulation become corpora lutea, others undergo follicular atresia), we find follicles that after the death of the ovum undergo a further follicle-cystic evolution: these are the follicular cysts and they constitute the most important anatomic alteration. That is, there are three types of follicles which simultaneously undergo different processes of evolution:

1. normal evolution: biphasic follicle → ovulation → corpus luteum corpus → albicans
2. normal evolution: monophasic follicle→atresia follicle→ corpus fibrosum
3. abnormal evolution: monophasic follicle→follicular cyst.

The monophasic and biphasic follicles are found together only exceptionally and transitorily in the metropathia hemorrhagica (in the form of Brennecke Schroeder disease) and in the sclerocystic ovary. That explains why the phenomenon that causes the definite establishment of these two syndromes has been inter-

preted as a dysfunction of the hypothalamus-hypophyseal-ovary system, that inhibits the action of the luteinizing hormone (L.H.) and consequently the follicles are checked in the process of maturation. They then suffer a cystic transformation and as a consequence the series of disturbances that characterize both syndromes.

But this explanation applies only to such diseases, as the metropathia hemorrhagica and the sclero-cystic ovary, of which the almost constant occurrence of monophasic follicles constitutes the pathogenic basis. On the contrary it does not fully account for the anatomical and functional syndrome of the polycystic ovary in the cancer of the breast with hyperestrinism, in which mono and biphasic follicles evolve simultaneously. Hence it appears that this phenomenon is not the result of a purely hormonal mechanism.

We must keep in mind that after differentiation and during the sexual lifetime the 300,000 to 400,000 primary follicles have two distinct functions: a) three to four hundred undergo the biphasic process: maturation, ovulation and transformation in corpus luteum (genetic function); that is to say, these follicles respond to the follicle stimulating hormone (F.S.H.), to the luteinizing hormone (L.H.) and to the luteotrophic hormone (L.T.H.); b) the others undergo the monophasic process of follicular atresia (vegetative and trophic functions), for they respond only to the follicle stimulating hormone (F.S.H.). Now, what mechanism regulates these two different destinies of the primary follicles? It is not yet known and we have to accept in theory that normally it must be determined in a prospective manner by a genetic factor which induces the formation of two types of follicles (biphasic and monophasic) which have complete and closed cycles, that is to say, maturation, development, involution and disappearance in a collagenic scar. This constitutional factor may be suspected of inducing the follicular cysts in patients with cancer of the breast and hyperestrinism. As a morphologic basis in support of the hypothesis of this constitutional factor we shall mention the curious malformation we have found in some ovaries: primary follicles in the initial stage of maturation with a dead ovum in the theca interna and externa layers (Fig. 6). It is likely that these ectopic follicles may undergo cystic transformation, but we do not suppose them to be

the origin of all the follicular cysts. If we consider the number of follicular cysts in each ovary (two to six) and that in their involution they do not disappear in a scar like a corpus fibrosum, we may assume that during the sexual life very few of the primary follicles become follicular cysts.

Briefly, there is a hereditary factor in the polycystic ovary of women with cancer of the breast and hyperestrinism which induces the formation of follicles according to natural laws, but temporarily deviates from normal (by a malformation or any other cause still unknown) and thus determines the development of follicles that evolve into estrogen-secreting cysts, which reach a state of partial involution without disappearing in a collagenic scar. They are, therefore, cysts of open cycle. In this way a real gland of continual secretion of estrogenic compounds is formed within the ovary. So, for years the mammary epithelium suffers the action of the estrogenic substances secreted by the cysts in addition to those secreted by the normal follicles during the sexual life.

III. Recapitulating the series of findings mentioned above we shall be able to fulfill the three objects contemplated in writing this paper. The fact that the ovary of these patients is a continual source of secretion of compounds of estrogenic action, accounts for the drop of the cornification curve below normal after bilateral ovariectomy and it shows that the ovary is the only source of estrogens. It also explains the temporary improvement observed after ovariectomy in some patients with hyperestrinism (arrest or decrease of the growth rate of the tumor, alleviation of pain and reduction of the oedema in erysipeloid cancer, regression of bone metastasis, general state of well-being) since the source of estrogens, in an estrogen-dependent cancer, has been removed. But if the curve of cornification is controlled for a certain time later, we find that after a variable period (three to twelve months) it rises gradually again to a new high percentage of cornified cells. This rise puts an end to the temporary improvement and marks a relapse into the neoplastic process. This second phase of the hyperestrinism is induced by the corticoadrenal gland, as demonstrated by the increase of the total 17-ketosteroids (Nathanson[11]) and by the new improvement obtained with bilateral adrenalectomy in patients on which ovariectomy had al-

ready been performed (Huggins and col.[7]). In this second phase the cancer of the breast becomes adreno-dependent.

The facts herein discussed show that in the ovary of these patients two factors of breast canceration act in conjunction, the hereditary or constitutional one, and the hormonal. We think that the former is the most important and that the latter should be considered as a consequence of it, the development of follicular cysts depends upon a genetic factor.

It would be of interest to establish if in other neoplastic diseases of the genital tract and also in women apparently normal, there are follicular cysts similar to those we have described. Anyway that would not change our point of view for we know that there must be other factors, and in particular a predisposition of the mammary tissue to canceration by the estrogens, and of course it is likely that in the absence of this predisposing factor the women with hyperestrinism are not in danger of cancer of the breast.

IV. We emphasize the hormonal action of the follicular cysts. Undoubtedly the cells of the granulosa layer and those of theca interna of the cyst wall continually secrete compounds with estrogenic action, but we cannot establish the amount in a unit of time. On the other hand, research should be carried on to study: 1) what estrogenic compounds are contained in the fluid; 2) if it contains other steroids. In fact, the multiple secretory capacity of the theca interna makes the answering of these questions a matter of great importance. Several authors (mentioned by Parkes[12]) have proved the secretion of androgens by the theca interna and the medullary part of the ovary. We have found different shapes of the cells in the walls of the cysts: simple hyperplasia, lutein cells and Leydig-like cells also, but rarely, foci of lutein-producing stromal cells. These histological aspects suggest that diverse and complex substances are possibly secreted. We believe that research in that direction will help to explain some of the phenomena observed in the patients of non-neoplastic secretory cysts (follicular and corpus luteum cysts) which have not yet been interpreted by us.

An important fact is that processes of inactivation and elimination act simultaneously with the ovary, thus resulting in the balance of the circulating estrogens. It is known that ten per cent of the estrogens are eliminated with the urine, mainly as metabolites, and that about ninety per cent are inactivated,

almost totally in the liver; perhaps a small amount in the spleen and in the kidneys (Paschkis and Rakoff[13]). It is generally admitted that the estrogen inactivating function of the liver is impaired in a variable degree, in the acute as well as in the chronic liver diseases, producing as a consequence a retention of those substances in circulation (Rakoff *et al.*[14]). Schiller and Pincus[15] demonstrated that the estrone injected in rats after partial hepatectomy retarded significantly its conversion into estriol. Therefore the alterations of the liver may cause the aggravation of a previous hyperestrinism. The records of our patients reveal a high percentage (seventy per cent) of complaints attributable to functional liver troubles. Thirty per cent had undergone operations for gall stones and the rest had constant digestive trouble after the intake of fats, particularly heated fats.

VI. As routine procedure the determination of the curve of vaginal cornified cells has the disadvantage of requiring twenty-six to twenty-eight days. In urgent cases, however, the curve of two weeks would suffice to obtain an estimate of the balance of the estrogens. For our studies it was very important to determine the curve during all the menstrual cycle, and it was done during this period in all our cases.

VII. Considering our findings, the prophylaxis of breast cancer based on its etiology offers one of the most promising prospects for a great number of women applying a simple control procedure. We ascribe great importance to the systematic determination of the balance of circulating estrogens (by means of the curve of cornified cells or, when not possible, the investigation in blood or in urine) in every woman with repeated troubles of the menstrual cycle. Whenever permanent hyperestrinism is established the diagnosis of functioning polycystic ovary syndrome must be ascertained. Palpation will very rarely reveal the abnormally enlarged ovary. So it would be advisable to perform exploratory laparotomy and proceed with partial resection of the cystic zones or the cauterization of the cysts "in situ." Considering that during the sexual life only a few follicles become functioning follicular cysts it will be possible to obtain the definitive cure of hyperestrinism. In this way the source of a continual hypersecretion of estrogens may be nullified and a possible canceration of the breast may be prevented when the mammary epithelium is particularly sensitive to the action of estrogens.

Summary

1. This paper deals with the clinical, hormonal and anatomo-pathological characteristics of a syndrome not as yet described, the *Functional Polycystic Ovary in Cancer of the Breast with Hyperestrinism.*

2. It contains a summary of the clinical, hormonal and anatomo-pathological investigations that served to establish its existence.

3. The knowledge of the functional polycystic ovary here described is interesting from two points of view: a) it gives an explanation of the role of the ovary in the genesis of hyperestrinism and in the canceration of the mammary epithelium; b) it offers precise bases to undertake the prophylaxis of cancer of the breast in a great number of women.

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THE DETERMINATION OF THE CANCER INCIDENCE IN THE COMMUNITY

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The Cancer Problem

Cancer is the second leading cause of death in the United States with morbidity data showing approximately 15 per cent or one person in seven dying of cancer, as reported by Dorn and Cutler (1955), Cannon (1954), Cutler and Haenszed (1954). There are an estimated 700,000 people under treatment for cancer and 500,000 new cases are expected each year. This means that more than 50,000,000 people now alive in the United States will have cancer at one time or another in their lives. Of these, 24,000,000 will die unless current methods of diagnosis, treatment and prevention are improved. This annual toll of 225,000 deaths due to cancer causes a staggering loss economically, both to the nation and to the families concerned.

Cancer is a group of many different diseases all of which share the common characteristic of individual cells of the body which are specifically and irreversibly modified and which leads to a common fatal ending. Certain cancers can be cured if localized and accessible to the surgeon or radiologist. However, many cancers, even if ordinarily accessible, are discovered too late for successful treatment. Cannon has discussed this recently.

Along with the obligation for medicine to diagnose and treat disease goes the complementary one of the prevention and control of the causes of disease. Research in cancer has many facets with basic work in the biology and chemistry of growth, improvement in surgical and radiological techniques, the use of hormones and other drug therapy, cytological studies and epidemiological studies.

This latter approach tries to assemble large numbers of cases of cancer in the search for the causal difference between those who have cancer and those who do not. These differences may lie in habits related to diet, sex, religion, hygiene, smoking, or even may be related to climate, geography, racial inheritance,

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specific genes or other unknown factors. By the statistical analyses of thousands of cases of cancer in large populations cancer morbidity trends may reveal information unobtainable in any other way.

Determination of the Incidence of Cancer

Ideally, the determination of the incidence of cancer seems a simple matter. However, until comparatively recently there have been few studies which could claim to give real incidence figures for cancer. Even the best current studies fail to give true incidence rates although they approach this goal more closely. This failure has been due both to the nature of the disease and the epidemiological methods of studying it. This is illustrated by Dorn (1944), Dorn and Cutler (1955), Cannon (1954), Cutler and Haenszed (1954), Lees and Lees (1950), and Gillian (1954).

The incidence of cancer may be defined as the number of cases of cancer first diagnosed during a given time of study, usually a year, per 100,000 population, including those cases revealed by death certificates only. Unless a study shares all the above characteristics it is not true incidence. A related concept of prevelance of cancer includes the total incidence of cancer plus all cases in the studies under treatment, but diagnosed in a prior year per 100,000 population.

Prior to the mortality survey of 1937 reported by Dorn, most statistical data utilized mortality records in an attempt to estimate the extent and frequency of cancer from such data. As the cure rate for cancer has advanced these estimates are known to be increasingly inaccurate. The increase in cancer due to an increasing aging population, or other factors, has brought an increase both in the incidence and prevalence of cancer not evident from mortality figures alone. This has been illustrated by Dorn and Cutler (1955), Cannon (1954), Cutler and Haenszed (1954), Lees and Lees (1950), Gillian (1954), Patno (1954), and Hoffman and Gillian (1954). Although the majority of morbidity data are taken from the vital statistics, studies by Lees and Lees (1950), Gillian (1954), James Patton and Heslin (1955) comparing the death certificates cause-of-death with

autopsy findings show how much in error or limited such statistics may be.

The diagnosis itself of cancer is not without certain difficulties. Other than histological appearance or the clinical assessment that a tumor is growing and spreading there is no specific laboratory or other standard test for cancer. Even the microscopic diagnosis of malignancy is open to a considerable amount of disagreement depending upon the site. With this latter in mind the fact that most cancer is symptomless therefore limits the study of the morbidity of cancer to an analysis of diagnosed cases.

At present there is no general method for determining the total number of people with cancer. Other than a complete general physical examination none of the more than 60 or more procedures suggested as diagnostic tests for cancer in the literature since 1930 have been shown to give a general specific test for cancer. Homburger has reported on Diagnostic Tests (1950). There are several avenues of approach which are useful for specific sites of cancer and other methods which may offer future success. Papanicolaou has evolved a cytological test for uterine cancer which depends upon a knowledge of the atypical architecture caused by the malignancy in the individual cell. Papanicolaou (1954) and Dunn and Sprunt (1955) have reported on cytology. This has made possible a possible diagnosis of a preinvasive stage of cancer, and in addition has made possible the recognition of cancer in a location, by means of detached cells carrying with them the histological appearance of their origin as well as their stigma of disease. This has been discussed by Homburger (1950) and Papanicolaou (1954). This new characteristic of neoplastic cells with altered cytological appearance is thus being used for diagnostic purposes. However, this test still depends upon adequately trained personnel as well as having limitations due to the fact that differences of opinion even among the experts may still exist.

The prohibitive cost of a complete general physical examination for any large groups of people and the difficulties of diagnosis mean that the true incidence of cancer in any of the previous surveys has not been attained.

Current Methods of Study

At the present time there are two general types of studies which attempt to determine the incidence of cancer within the

limitation of materials and methods currently available. This is illustrated in the papers by Hoffman and Gillian (1954), Homburger (1950), Burdick and Chanatry (1954), Dunn and Sprunt (1955), Robbins *et al.* (1953) and Cobb *et al.* (1954).

1. *The case history or indirect method.* This method utilizes the time-honored procedure of grouping cases together with a similar disease. The characteristics of the patient are noted either from direct observation or by interview. These data are compared with a control group that is well, or with a group with a clinically unrelated disease. The risk to disease is thus inferred from the differences demonstrated between the study and the control group. Data on the age, race, sex, marital status, occupation, family history, place of residence, etc., are recorded. Associations are extended to large series and localities including general hospitals to obtain sufficient numbers for statistical significance. Questionnaires may be distributed to increasing numbers of widely scattered physicians, including those specializing in specific diseases. When great care is taken in selecting the patients and the control groups this type of study may represent history taking at its best for many types of surveys. It has limitations for many types of cancer in the security of the evidence obtained as will be discussed later.

Modifications of case history may be such that instead of incidence in a given locality the occupational or racial incidence may be employed. This is regarded as appropriate to diseases requiring specialized skills for diagnoses. Since the autopsy record is regarded by some as the only evidence of diagnosis a series of necropsies reports has been often taken as the point of departure for computing percentages that the disease is in the total population.

This method is not incidence unless certain conditions are fulfilled. These include the fact that all illnesses diagnosed in the hospital are included, as well as the diseases under consideration. With autopsy series alone the selective factor operating in bringing the patient to the hospital in the first place, as well as those operating in the decision as to which fatal case is autopsied, question seriously, if not makes worthless any inference as to true incidence from such figures.

Autopsies in most hospitals represent a sample of an unknown amount of illness occurring in a population of unknown compo-

sition. No one has yet devised a practical, uniform way to compute incidence from data of this kind. Thus the case history method or indirect method although it may give accurate observations of the cases studied, is inadequate from the viewpoint that selective factors operate to decrease the reliability of any statistics as applied to the general population as regards the true incidence of the disease under study. However until simpler and more reliable methods are evolved surveys will continue to be studied in this manner. By understanding their limitation facts which can be deduced from such studies are put in their truer perspective.

2. *The population or direct method.* This method has as its point of departure a group of people instead of a group of cases of a given disease. Then disease occurrence is measured in various segments of the group. This may be done more or less in retrospect. However, it is preferable that the population be first characterized and then the subsequent occurrence of disease in subgroups with different characteristics is measured by means of a study projected into the future. This is thus the ideal way to measure incidence of any given disease. However, due to the magnitude of the task, time required, and expense involved, many surveys must of necessity be more modest in their approach.

Sources Available and Their Accuracy

As already indicated there are innumerable articles available, including those by Hoffman and Gillian (1954), Shimkin (1954), Watson (1953), Mider *et al.* (1952), Shimkin *et al.* (1954), Burdick (1954), Dunn *et al.* (1955), utilizing the case history method for determining the incidence of cancer in the general population. Some are more accurate than others in their presentation of the objective findings. Lees and Lees (1950), Gillian (1954), and Homburger (1950) can be referred to in this respect. They all suffer however from certain selective factors which make it impossible to accurately estimate the true incidence of disease in the general population.

The security of evidence obtained by either the case history or the population methods varies with the disease under study. The characteristic under investigation, particularly the likelihood of

it being remembered and divulged with equal accuracy by the control cases is difficult in cancer surveys. This reticence particularly about cancer makes the interview method alone unsatisfactory as regards control subjects as well as subjects with cancer. Thus the security of evidence by the interview method is subject to question. Sampling cases and controls may have certain selectives not immediately apparent although it is not too difficult to obtain representative groups as regards age, race, sex and residence. In many instances until the information is accumulated one may be unable to estimate accurately just how representative the sample is.

If the data obtained are looked upon as initial validity comparable to a clinical impression they are invaluable in providing concrete leads and points of departure for future investigations. They should not be regarded as concrete fact until sufficient direct and indirect verification attest to their consistency.

The difficulties of dividing the population into those who do have the disease and those who do not depends upon the ease with which the disease may be accurately diagnosed and the ease in counting the cases once they have been clinically identified. In cancer the precision of diagnosis depends upon the kinds and availability of diagnostic skills and techniques for effective identification. The average practitioner can many times make an accurate diagnosis on clinical grounds. However special care, hospitalization, laboratory procedures, and finally autopsies all decrease appreciably the number of cases unrecognized. Between the two groups all gradations in diagnosis are encountered.

It would be very helpful if there were developed some inexpensive and objective diagnostic tests applicable to the general population for the diagnosis of cancer. All science seeks is increase in refinement of measurement. Certainly the autopsy report on a relative scale is much more accurate than the death certificate. If there is adequate understanding that autopsy rates do not mean incidence due to the extreme selective factors operating necropsies can be used to refine the death certificates in the area served by the hospital. In this, accumulation of vital statistics has an added refinement in measurement.

Case registers for cancer for epidemiological purposes require competent statistical design and close technical supervision which is expensive. Therefore for most purposes case registers

serve the purpose of directing attention to the service offered. As most registers operate they are unrepresentative of all existing cases as are those cases which gravitate to some particular hospital.

Until adequate access to noncummunicable disease as it occurs in definable populations is available, substitute procedures for estimating their number and characteristics will continue to be employed. Thus the large hospital will continue to use both the case history and autopsy reports. The fact that there is no practical way to make the material of more general usefulness in epidemiology is a challenge.

There is one elaborate group of cancer morbidity surveys which utilize the population of direct method of the study of incidence. The reports by Dorn (1944), Dorn and Cutler (1955), and Patno (1954) are illustrative. Ten metropolitan areas in two separate surveys separated by an interval of ten years (1937–39, 1947–49) had a statistical analysis of thousands of cases of cancer in relation to the population and the cancer morbidity trends were determined. As already mentioned there is no known method to estimate the true incidence of cancer. Morbidity studies in cancer are therefore confined to an analysis of diagnosed cases.

The areas included are Atlanta, Birmingham, Dallas, New Orleans, San Francisco, Denver, Colorado, Detroit, Philadelphia and Pittsburgh. The information obtained included the resident cancer patients diagnosed, treated or observed during a 12 month period by all hospitals, clinics, and physicians in each area surveyed. This information is related to population data of the pertinent area involved and the rates computed. The 1937 survey reported by Dorn (1944) was the first large attempt to measure cancer in the United States. The 1948 survey reported by Dorn and Cutler (1955), and Patno (1954), evaluated the changes occurring since the previous survey.

The illness rates included the incidence of the number of cases of cancer first diagnosed during the year studied per 100,000 population, including cases revealed by death certificates only. Cancer cases diagnosed and treated the previous year and presumably cured were under observation, not treatment. Prevalence rates are also recorded for all the same categories. Prevalence rates were 437/100,000 in 1937 with an incidence rate of

295/100,000. In 1947 the prevalence rate was 500/100,000 population and the incidence rate was 371/100,000. With increasing diagnostic techniques and case finding methods it can not be determined from the available data whether the apparent increase in the ten-year period for both prevalence and incidence rates is real or only due to other factors, known or unknown.

The morbidity data from any one hospital can not be as broadly interpreted as when the illness data obtained for a specific geographical area are related to known population groups such as were done in these surveys, by Dorn (1944), Dorn and Cutler (1955) and Patno (1954). However, comparison of a smaller and even restricted group with this large survey would certainly bring into focus any outstanding and large differences which might be explained by selective factors, probably, or other reasons. The collection of this data on a confidential report form on each patient treated, diagnosed or observed and in 1947 the patient being alive gives a further refinement to the older use of mortality tables or vital statistics for estimation of incidence rates. Whether there will be any real and useful correlation between the morbidity data and vital statistics remains to be seen when the summary is available for study.

The extent of these surveys make the comparison of conclusions reached here with other surveys of great importance. Survival is proportional to the stage at diagnosis and the current statistics show a diagnosis of 60% when still localized. The number undiagnosed and therefore untreated decreases with the alertness of physicians and an increased awareness of cancer in the general population. The positive correlation of cancer incidence and chronological age comes as no surprise, since more than 50% of diagnoses are in the 50 to 70 year age group. There is an increased variation between males and females relative to the parts of the body and the age cancer manifests itself. In the female 50% of cancer is in the reproductive system and 25% in the digestive system whereas, in the male, there is only ⅛ in the reproductive system and ⅓ in the digestive tract. Although the early diagnosis of cancer depends upon the awareness of an alert physician and an awareness of cancer by the general population, both these statements need further comment. It has been reported, for example, by Robbins *et al.* (1953), that physicians themselves are no better than the general population in early

diagnosis of cancer. Cobb, Clark *et al.* (1954) have noted that the information about cancer should be designed to communicate with the greatest number likely to cause delay in diagnosis, namely older people with little education. Although information about cancer is a potent weapon, the periodic health examination is still the best educational medicine at the disposal of the physician.

As to the reasons given why physicians are not concerned with early diagnosis in their own persons and why they permit unjustifiable delay before taking curative steps, they make no more sense than the reluctance of the ordinary layman. They also seem to choose as the initial consultant another physician with a culpability for delay as great as the general practitioner.

An interesting study on the incidence of multiple cancer in the same individual shows two studies of diametrically opposite conclusions. Watson (1953) concluded that the incidence of two or more multiple cancers does not differ from the ordinary incidence rate of cancer in the normal population. No evidence was found either of constitutional tendency or of the development of immunity following the first cancer. The study by Mider Schilling *et al.* (1952) suggests that the number of multiple cancers compared with expected incidence is greater than expected to occur by chance. It is obvious that neither study has true incidence figures and that factors of selection operated to cause different conclusions.

With all the comments presented upon the accuracies or inaccuracies in cancer morbidity statistics certain comments about the inaccuracies in the date of cause of death as compared to autopsies performed is not inappropriate. The study and suggestions by James, Patton and Heslin (1955) are illustrative in this respect.

The inaccuracies in the cause of death occur:

1. The physician does not list all available material correctly on the death certificate.

2. Clinical and laboratory data available before autopsy are not sufficient to enable the physician to determine the cause of death correctly.

3. Some cases with all the data available including an autopsy report still cannot decide the underlying cause of death with certainty.

Suggestions for improving these difficulties include:

1. Send post-mortem data with autopsy report to local health bureau.
2. Uniformity of reporting.
3. The problem of multiple causation remains. By recognizing its complexity perhaps the number of unknown cases will gradually decrease.

Thus two of the main problems in cancer morbidity data have solutions not impossible of improvement. The increase in diagnostic recognition and treatment of cancer can be improved. By estimating the incidence and prevalence rates in large areas some of the epidemiological evidence may reveal possibility of predicting and preventing cancer.

Ways Surveys Might Be Used to Indicate Incidence of Cancer Locally

As already mentioned autopsy data alone cannot by any known method be used to indicate the incidence of cancer locally. However, if statistics on other diseases in the same hospital, or better yet, the same geographical area are also available, these data may be used with increasing validity to estimate incidence with the limitations and reservations already mentioned.

Comparison of these data with data of similar nature in the surveys mentioned (Dorn, 1944; Dorn and Cutler, 1955; and Patno, 1954), might well be used to indicate the incidence of cancer locally.

Conclusions Regarding Actual Incidence of Cancer Locally

By comparing these data with those in the survey by Dorn and Cutler (1955), differences in actual data in the same years and trends can be noted.

No conclusions regarding actual incidence can be made without the data, method of collection and other information.

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POLYMETACHROMATIC STAINING OF PARENCHYMAL AND STROMATIC COMPONENTS

C. DE CHENAR*

Histopathological observations on regressive changes in degenerative, inflammatory and necrobiotic processes, furthermore on progressive, reparative and neoplastic phenomena, converged on the necessity for a flexible staining method to follow up these alterations from the earliest to the final stages of their development.

The fully developed degenerative and necrobiotic phenomena are clearly demonstrated by numerous standard techniques, with their more or less specific, or at least characteristic affinity to certain anionic or cationic dyes. The application of the principles of metachromasia[5] and allochromasia[15] yields a wider range of contradistinction between physiologically unchanged and pathological elements. The fact that certain tissue components are stained by certain dyes in a color shade different from that of the staining solution, however, has its limitations due to the molecular structure of the cellular and interstitial substrate and to its chromotropic properties, besides the decisive role of the dye itself.

Metachromasia occurs in both vitally stained and in previously fixed materials, with both basic and acid dyes.[11] The essential variables, parameters, which determine the metachromatic reaction are the concentration, dielectric constant, pH, ionic strength and temperature.[3, 9, 13, 15] The reaction might be regarded as a specific association between certain tissue components and a suitable dye. The latest interpretation of the phenomenon explains its mechanism as an electrovalent bond between substrate and dye, as a primary dye-chromotropic bond, plus another bond between adjoining dye molecules.[3]

The development of metachromasia, a gradual deviation from the original color, might be studied in solutions, without the participation of a preformed, organic, chromotropic substrate.[9] This change in color is based upon the presence of an unsaturated

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amino group and upon the possibility to produce an imino base. This phenomenon, however, shows chemical and spectroscopical characteristics considerably different from that of the genuine metachromasia. The parametric variables, as the increase of dye concentration or of the pH, as well as the decrease of temperature, favorably promote also this reaction. The addition of a minimal amount of chromotropic substances precipitates the process. The addition of electrolytes and aceton has the opposite influence upon its development.[4]

Although "in vitro" studies did result in many essential data concerning the principles of metachromasia, "in texto" experiments have disclosed its fundamental facts, and the limitations of its practical application.

The feasibility of metachromatic reactions is primarily bound to the molecular structure of the dye applied, if the parametric variables are properly aligned upon a suitable substrate. Acid dyes are bound strongly at low pH, moderately at intermediary values and not at all in alkaline media. In case of basic dyes a reverse attitude may be observed.[4, 8, 10, 11, 12, 13, 14, 15] The maximal binding of acid dyes occurs around pH 2, while that of basic dyes around pH 11.

Metachromatic staining of various tissue elements or that of their metabolistic and degenerative derivatives might be obtained at optimal pH level with a series of basic dyes as neutral red, thionine, toluidine blue and other phenothiazine compounds, but also with acid dyes as the group of Congo red.[9]

The aim of the present work is to develop a multiple metachromatic reaction for the one slide demonstration of regressive or progressive changes. The method described below is one of those developed over a period of years in this laboratory. Its basic principle is to trace tissue components of certain chromotropic affinity by staining those in colors and nuances clearly different from the non-pathological variants and from elements undergoing another pathological change. In a given battery of staining and differentiating or mordanting solutions, the materials are exposed to alternating pH differences which might control both the chromato-chromotropic and the interchromatic bonds, provided that the rest of possible parametric variants is favorably arranged.

Staining Method

Neutral or buffered 10% formalin and 95% ethyl-alcohol are the most suitable fixatives. Both celloidin and paraffin sections, 10 to 20 μ thick, may be used. The room temperature is preferably between 20° C and 25° C.

1.	Ammonium persulfate, 0.5% in distilled water. Depending upon the degree of mordanting desirable		10–60 sec.
2.	Distilled water		2 sec.
3.	Acid fuchsin	0.5 gm	
	Basic fuchsin	0.5 gm	
	Safranin O	0.2 gm	
	Distilled water	100.0 ml	20 sec.
	(Filter before use)		
4.	Blot dry.		
5.	Phosphotungstic acid	0.5 gm	
	Phosphomolybdic acid	0.5 gm	
	Distilled water	100 ml	
	(Filter before use)		20 sec.
6.	Blot dry.		
7.	Orange G	2.5 gm	
	Methylene blue—azure	0.2 gm	
	Aniline blue	1.0 gm	
	Oxalic acid	3.5 gm	
	Distilled water	100 ml	
	Add ingredients to boiling water, cool, filter and add 2 drops of concentrated HCl. (Filter before use)		30 sec.
8.	Blot dry.		
9.	Ammonium hydroxyde in distilled water, at pH 10 (appr. 0.25%)		dip 1–10x.
10.	Hydrochloric acid in 70% ethylalcohol, at pH 1.7 (appr. 0.75%)		dip 1– 4x.
	The differentiation in these solutions is rapidly completed. As soon as the material takes a pale greyish purple or pinkish purple shade, depending upon the tissue, and it becomes transparent before a white background, dip the slide immediately in the acid solution and shake in it, for a time proportional to that in the alkaline solution.		
11.	Distilled water. Dip several times, until the entire surface of the material becomes uniformly hydrated and smoothly covered by the water.		
12.	Back to solution No. 7,		5 sec.
13.	Blot dry.		
14.	Ammonium molybdate, 0.2% in distilled water		1 sec.
15.	Blot dry immediately.		
16.	Ethylalcohol, 70%		dip 2–4x.

Differentiation is completed as soon as the alcohol dropping from the slide is colored and the tissue is transparent.

17. Ethylalcohol, 95%, quickly move to
18. Ethylalcohol, absolute.
19. Xylol, 3 changes, the last one neutral
20. Canada balsam or a neutral mounting medium.

The color shades obtained this way are stable for years. One precaution, however, has to be advised, namely, if the slides are used for projection in carbon arc projectors, within a few minutes, gas formation results, with permanent damage to the material.

Selected Fields of Application

The following photomicrographs are an attempt to represent in black and white a few typical cases. Obviously, the contrast of bright colors is entirely lost and only differences in the density of the stained material are reproduced.

(1) Senile involution of the brain cortex and subcortical ganglion cells. The perikaryon and the dendritic processes contain dark blue or purplish blue tigroid granules and fragments of purple mitochondria. The lipofuscin is unstained. The nuclear chromatin substance is deep blue, the nucleolus is brilliant red. In advanced cases with tigrolysis, the protoplasm takes a light yellow shade. Chromatolytic nuclei stain greenish blue. Karyolytic and karyorrhectic nucleoli stain greenish blue and advance toward bluish brown. The purplish blue staining of non-myelinated fibers is in sharp contrast against the yellowish red shade of myelinated fibers. In an extreme case, a man of 108 years, the protoplasm takes an intensively bright red staining in cells undergoing pyknosis and pigment atrophy. No other staining method indicated any similar differentiation in these cells. Senile plaques are bluish brown.

(2) Arteriosclerosis. The earliest, pre-sclerotic degeneration which is not demonstrated by routine methods, is clearly shown in a bright yellowish red shade against the bluish-purple tunica media. The elastic membranes are sharply drawn in brown. In advancing scleratheromatosis, yellowish or greenish brown amorphous, granular or dust like deposits appear in the patches.

(3) Perivascular edema. In case of death ensued after electro convulsive therapy, in scattered areas of the cerebral, cerebellar cortex and white substance, there are maximally filled pre-

capillaries and capillaries. Many of those are ruptured. In the areas of paralytic vasodilatation, perivascular edema developed, resulting in semitransparency of the otherwise clear interstitial spaces and in distinctive discoloration of the neuroparenchyma. Both myelinated and non-myelinated fibers take a bright yellow staining, with gradual restoration of the original color toward the periphery.

(4) Subarachnoideal hemorrhage surrounding an "aneurysma mycoticoembolicum ruptum." Case of hematogenous dissemination of pulmonary abscess in a subarachnoideal artery. The purulent periangiitis shows various shades of dark brown and purplish areas. The corpuscular elements which penetrate into the cerebral cortex change their color from dark red to red, yellow and greenish yellow, due to their progressive disintegration.

(5) Gliosis, following encephalomalacia, vulneration of the brain and lobotomy. The proliferation of protoplasmic or fibrous astrocytes is nearly electively demonstrated. They have a lighter or darker blue cytoplasm, light brown nucleus and vivid red nucleolus. Micromesoglia cells show their brown reticular structure on a greenish yellow background. Blood vessels, including capillaries, take a sky blue staining. Erythrocytes stain lighting red, leukocytes and lymphocytes are yellow, with brown or deep blue granules.

(6) Meningoma, cytoblastic or fibroblastic. This is a very excellent material for the study of degenerative changes. The parenchyma is light blue or greenish blue. As the fibers are formed, they change toward yellowish green. The psammoma bodies, in the early stages of their development, are greenish yellow or light yellow. As their lamellation begins, they stain dark or brownish yellow. Many of them show brilliant reddish yellow granules between the concentric brown rings. Those with far advanced changes are dark brown with a purplish shade. Areas undergoing central necrosis change to purplish red.

(7) Glioblastoma multiforme. This is another suitable subject. The cytoplasm of the cells in palisade formations stains light or dark blue, their nucleus light brown. In cells around necrotic areas the nucleus is red or yellow, their chromatin substance is bright red, scattered in fragments or conglomerates. This method shows a consistent change in the surrounding neuro-

parenchyma, bright red, globular granules of equal size both in the perikaryon and in the dendritic processes at their root. Routine staining methods do not demonstrate these bodies.

(8) Squamous cell carcinoma of the cervix uteri. The cytoplasm of tumor cells is either blue or purple, their nucleus is greyish yellow. In various stages of the pearl formation, the appearance of keratohyaline is indicated by a yellow or brownish yellow shade. A similar but faint discoloration might be observed in areas which are about to turn into pearl formation.

(9) In cases of fibrinous pneumonia a rather unique phenomenon was found. Besides characteristic color differences in the involved pulmonary parenchyma and in the infiltrating components of hepatization, the precipitated fibrin stains sky blue or darker blue within the inflammatory process in the alveoli—but it shows a distinctly different staining within the coagulated blood plasma in perivascular spaces. Here, it takes a clearly red, often bright red staining. This fact indicates that the inflammatory and the coagulative fibrin, as chromatropic substrates, represent two definitely different structures.

Comment

The principle of polymetachromasia has been developed. In a battery of mordanting, staining and differentiating solutions, the materials are exposed to alternating pH differences, while the rest of the possible parametric variants, in a favorable arrangement, is constant. The time in the differentiating solutions determines, in a flexible way, the qualitative and quantitative extent of possible chromato-chromotropic and interchromatic bonds, *i.e.*, metachromatic staining. Experimentally, a series of staining methods with both anionic and cationic aminobenzol derivatives was applied for the demonstration of mainly regressive phenomena of various causal etiology. Not only complete degenerations but quasi pre-degenerative conditions were observed in cases of scleratheromatosis, perivascular edema, homoiotypic and pseudoplasmatic tumors, as well as in physiologically far advanced senile involution. Further investigation is being carried out on the differentiation of fibrin coagulated in inflammatory processes and that found precipitated in perivascular edema, as they appear to have characteristic chromotropic affinity. The

staining method outlined above has disclosed several facts from a new angle. Modifications, other procedures and multiple variations in parametric factors are in process for the elective demonstration of karyokinetic phenomena in tumors.

Summary

A histopathological staining method, based on the principle of "polymetachromasia" is presented. It is sensitive for earliest regressive changes, as well as for advanced necrobiotic processes particularly in tumors. Both parenchymal and stromatic phenomena are clearly demonstrated. The technique is rather simple, however, longer experience is a prerequisite for the flexible control of the polymetachromatic or polymetachromatoid staining.

Dr. Sam A. Hoerster, Jr., Superintendent, and Dr. S. W. Bohls, President of the Texas Society of Pathologists, gave helpful encouragement and support.

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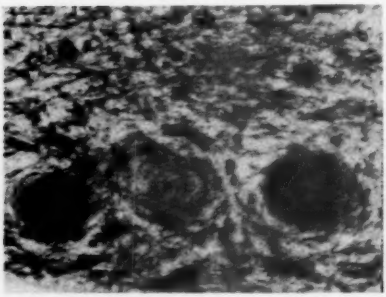

FIG. 1a. Senile involution in cells of the nucleus emboliformis. Age 108.

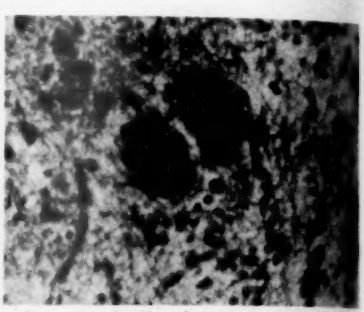

FIG. 1b. Senile plaques in area of 8 Brodman, gyr. front. med. Age 108.

FIG. 2. Moderate arteriosclerosis in the art. meningea media. Age 53.

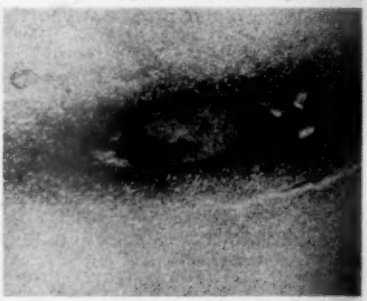

FIG. 3. Perviscular edema in the pons.

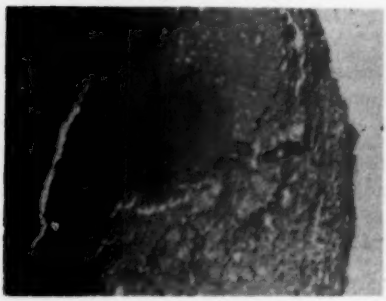

FIG. 4. Subarachnoidal hemorrhage around a ruptured mycoticoembolic aneurysm.

FIG. 5. Gliosis, following lobotomy.

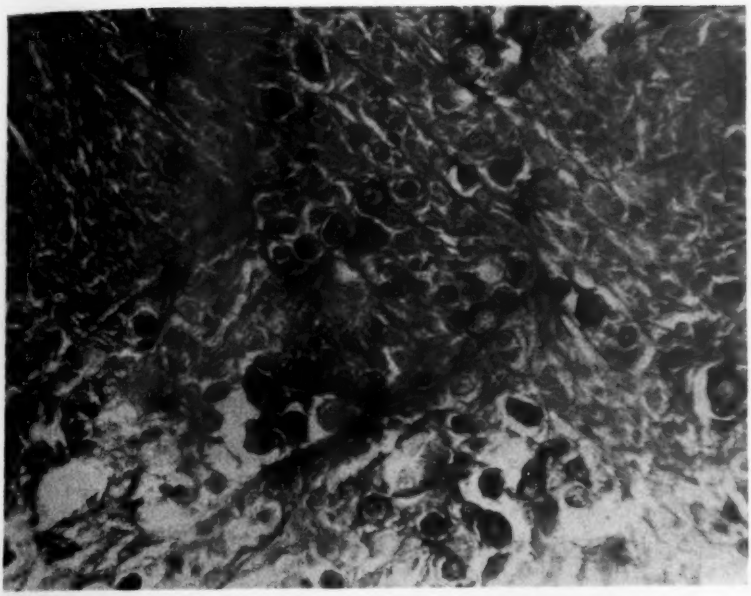

FIG. 6. Meningoma, parasagittal supraethmoideal. Psammoma bodies in various stages of development.

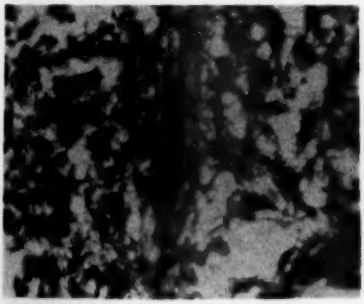

FIG. 7a. Glioblastoma multiforme. Perivascular formation.

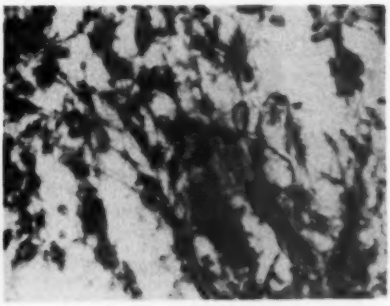

FIG. 7b. Glioblastoma multiforme. Palisade formation.

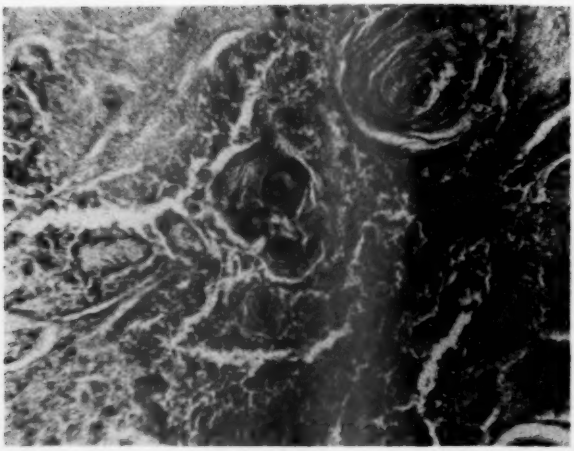

FIG. 8. Squamous cell carcinoma of the cervix uteri Pearl formations.

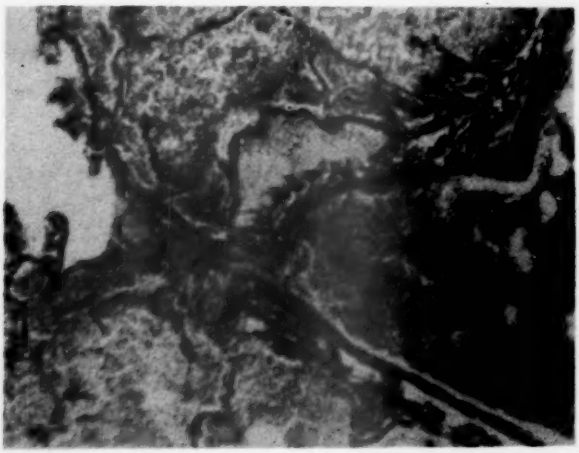

FIG. 9. Fibrinous pneumonia.

THE PERSISTENCE (SURVIVAL) OF MICROORGANISMS: IV. IN FOOD

FRANK B. ENGLEY, JR.*

One of the main avenues of invasion of disease producing organisms and toxins is by the oral route. Many, but not all, pathogens can infect this way and not all of those that enter by that route are true intestinal pathogens in the strict sense of the word. Poliomyelitis virus would be an example.

Food such as green vegetables and fruit may get contaminated in the gardens from fecal material or handling. If eaten raw without proper washing the organisms of typhoid, dysentery and certain parasites of protozoa and worm types may cause infection.

One of the major methods by which pathogens get into food is by improper, unsanitary methods of handling. In one way or another, the organism in fecal material or other excreta get from humans or animals into the food. Very few withstand mild heat. Proper cooking would destroy all of the potential pathogens.

In some instances, the pathogens are present in animal materials used as food. That is the milk, milk products or meat may contain animal pathogens with which the animal is infected. Some of these pathogens may also infect humans. If the organisms persist in the milk products which are not treated by pasteurization (milk) or proper cooking as in the case of meat, the infection may occur. Sometimes the pathogens transmitted by the milk or meat are from the food handlers and not from the animals at all.

A few of the potential pathogens may actually grow in the food. This increase in numbers of organisms may result in such numbers as to cause "food poisoning" when eaten. Examples would be Salmonella organisms. A few such as the Staphylococcus and *Cl. botulinum* organisms produce toxins in the food which also cause "food poisoning."

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The factors affecting survival of organisms in food may be grouped as to (1) food factors affecting and (2) organism factors affecting.

Food Factors Affecting: The general type of food product will affect the survival of any organism in it. Particular characteristics would be: (1) amount of moisture present to allow or prevent multiplication of the organism; (2) presence or absence of antagonistic organisms; (3) the use of the food by the organism as nutrient increasing the number and for producing detrimental end-products for survival; (4) the pH of the food; (5) the presence of inhibiting quantities of sugar, salt, spices or other preservatives; (6) the temperature under which it is stored; (7) if frozen, the rate of freezing, and (8) the amount of light or radiation.

Organism Factors Affecting: An organism may survive or persist in foods for varying lengths of time depending upon (1) the inherent resistance of the genus and species under test; (2) the particular strain of organism studied; (3) the presence of a protective covering on the cell, such as a capsule; (5) the age (in the growth curve) of the inoculum; (6) the numbers of organisms inoculated; (7) the ability of the organism to multiply in the food under the conditions presented.

Summary

**Bacillus* (Table F1) *Bacillus anthracis* was found to survive in milk for 10 years, was present on oats and on corn roots for 50 days and beans for 6–11 days. Other Bacillus species were found commonly in margarine and to survive on fruit for long periods at low temperatures.

**Brucella* (Table F2) In milk the bovine strain lived for 5–10 days. In sheep milk persistence of 22–40 days was observed at refrigerator temperatures. In dairy products such as butter, 142 days survival and with cheese, 1–2 months most common but as long as 1 year was listed. Ice cream kept for 5–7 years at —23 C. still had Brucella present. In unsmoked ham survival of 21 days was reported. Brucella survive for an hour in wines and up to 3 days in beer.

**Clostridium* (Table F3) The organisms are present in cheese and other milk products, on vegetables, meats and on fish, usually in the spore state.

Clostridium botulinum was able to exist for over 2 years on vegetables at —16 C. as well as a large variety of foods at similar temperatures. Low pH inhibits the organisms as does high temperature.

**Coliforms* (Table F4) The organism may be present in milk and dairy products for long periods depending on pH and temperature. Some cheeses harbor it for 12 months or more. In frozen eggs *E. coli* recovered, even after 5 years.

It is present in sea foods and may live in sausage for 13 days. Vegetable surfaces may have the organism on for long periods. Storage at —4 F. allowed survival for a year. Fruit surfaces are also contaminated and can live for 2–4 months at low temperatures. Beverages such as milk and beer may have the organism present.

**Micrococcus* (Table F5) Micrococcus species were present in milk, eggs, meat, sauces, on vegetables and fruits. Some 56% of margarine samples had Micrococcus species present. In eggs at —9 C. survival for 1 year is listed. In meat, survival of over 60 days at 22–37 C. was reported. Some survival of 16–144 hours in mayonnaise was found even at low pH. On vegetables at —17 C. 8 month survival is reported. Fruit at —18 C. contained organisms for 6 months while juices at —4 C. lost viable counts at 50 hours.

**Microorganisms* (Table F6)

Corynebacterium—In frozen cream for over 4 days, butter for 1 month and sausages for 24 hours.

Lactobacillus—Present in milk for long periods and on peas for over 2 years at 15 F.; in butter for 275–462 days.

Rickettsia—In milk for at least 24 hours and probably 7–30 days. Cheese for 46 days and butter for 41 days.

Achromobacter—Viable in butter for 239 days.

Bacterium linens—4 months survival in cheddar cheese at low temperature and pH.

Trichinella—In pork, survival for a few minutes to 36 hours at sub-freezing temperatures.

Pasteurella tularensis—Present in grain and food contaminated with rat excreta.

Pseudomonas—On plants for 69 days.

Proteus—In fruit juices at sub-freezing temperature for almost a month.

**Microorganisms (General)* (Table F7) The general effects of low temperature in keeping bacterial flora of milk and milk products low are given. Similar reports are given for eggs. Temperature and humidity effects on survival in meat are shown as well as temperature reduction of organisms in fish. Organisms were present in frozen vegetables for over 4 years at —18 C. Temperatures of 65–80 C. did not destroy organisms. Low temperature and moist soil plus organic matter allow pathogens to survive. On fruit surfaces many organisms may be present. They survive at low temperature for long periods up to 3 years. Low pH destroys them rapidly.

**Mycobacterium tuberculosis* (Table F8) In milk, survival of 10 days to 2 years is listed. Low temperature allows long survival. Sour milk destroys organism. Temperatures of 60–80 C. kill rapidly. In dairy products at 15–22 C., 2–30 day viability is listed depending upon pH. Cheese may harbor the organism for 2 weeks to nearly a year depending on type (of cheese). Ice cream kept for 4–6½ years yielded live organisms. The organism survived pasteurization in butter. Several reports on food suggest that fat protects organisms.

**Salmonella species* (Table F9) The major proportion of reports on organisms in food dwells on Salmonella species. In milk, Salmonella types may survive

at refrigerator temperatures about 170–324 days. Many experiments with lowered pH show decreased survival. At pH 4.7 for 11–63 days and pH 4.2, no survival. Different species and strains vary as to sensitivity to acid. Sterile milk plus Salmonella give food survival suggesting antagonism may destroy them. In dairy products such as butter 117 days survival is suggested, for curds only 48–96 hours, for ice cream about 6–7 years at —23 C., in butter 49 to 212 days, buttermilk for 10–15 days, cheese for 24–30 days or even to 6–10 months, depending on the species and inoculum size.

In eggs at —1 to —18 C. some 11 months survival reported, while dried egg at 35 F. allowed viable forms at 65 weeks. Egg albumin was contaminated almost always and the organisms lived for 20 days at 120 F.

In meats of all types, Salmonella were present in some 1 to 26% of samples. In chicken at —25 C., 270 day survival is listed. In corned beef, 60 day viability is given. In oysters, 4 to 24 day survival is given. In other sea foods 4–40 day survival was found at low temperature.

In sauces such as salad dressing, survival of 1 to 144 hours was found. In cereals and breads 4½–6 month survival. On vegetables Salmonella may stay for a few days to several weeks at body and refrigerator temperatures or up to 25–31 days at room temperature. Several reports suggest 200 day-3 year persistence in canned vegetables. In frozen foods, 12 week survival. On greens for salad, 12 hour to 21 day survival has been observed. In or on fruit these organisms live for a few days in low pH juices or as long as 1–3 months in frozen fruit or for 68 days on surfaces. In beverages survival of Salmonella may be for 38 days or more in cold beer or for 1 hour in wine.

**Shigella species* (Table F10) In milk at refrigerator temperature, 18–27 day survival is listed with 53 days in pH 4.8 milk but only 3 days at lower pH. In milk products survival varies from curds with no survival to butter at 18 day viability. Cheese may harbor the Shigella for 9 days. Eggs may be contaminated for 3 months at —9 C. Meats contain viable organisms for over 3 weeks. Cereals and bread may have Shigella present for 1 day up to 45 days with decreasing temperature. Fruit have been contaminated for 2–10 days.

**Streptococcus species* (Table F11) Some of these organisms are quite common in milk, but length of survival apparently is not too long. Reports suggest 8–48 hours in fresh and sterilized milks. In dairy products pyogenic streptococci have been found for varying periods such as in cheese from 9 days to over 18 weeks depending on type of cheese and in butter for 17 days. Other streptococci may exist for 6 months in butter. Ice cream may be contaminated for 12 hours to 18 days. Eggs may contain these organisms for a few hours while meats have been harboring them for 13–60 days. Frozen vegetables may have streptococci in them for a year.

**Vibrio species* (Table F12) The cholera organism survives in milk for short periods of a few hours at room temperature to 8 days in sterilized milk. In dairy products such as butter, viable organisms have been found for 21-over 98 days with low temperatures extending the period of survival. Cheese does not allow very long survival with reports varying from 8 hours to 4–5 weeks. Curds and whey showed poor survival. In fish eggs, the vibrios lived from 12 hours to over 8 days at low temperatures. The organisms were present in

various fish and meat preparations for varying periods. In fish, the usual survival was for a few hours to a few days. When salted and/or at low temperature low viability was found in fish. In meat, a report of 45 weeks was found but another report suggested 2 weeks at 3–8 C. and 7–10 days in hot weather. The vibrios survive in sauces from 1 hour to about 24 hours. In cereals, 8–15 hours and on vegetables, for 4–5 weeks; on fruit as long as 4 days are also reported.

•*Viruses* (Table F13)

Poliomyelitis—The virus resists heat in milk better than in water. In butter, 91 days viability was observed. The virus was found on fresh fruit and vegetables.

Foot and Mouth—The virus has been found in milk. It exists in beef at —4 C. for 24 hours and at —20 C. for 4 months. Some reports of its presence on cereals suggest 4–20 week persistence.

Newcastle—In eggs, survival of 126–538 days is listed. At 36 C. over 100 days and at 3–6 C. over 500 day survival was found. In mash, 56 to over 538 day viability was observed. At pH 5 and 37 C. 56 days was the extent of persistence while at pH 5 and 3–6 C. over 500 day existence of virus was observed.

Fowl Pox—In dried eggs, survival of 1928–3598 days (10 years) is reported.

Encephalitis, Jap B—In eggs at 4 C., only 6 hour survival was found.

Pigeon Pox—In dried eggs, viability of 1099–3605 days has been demonstrated.

•*Yeast and Mold* (Table F14) Yeasts are reported in milk and in margarine (46% of samples). Vegetables contain yeasts after being frozen but 90% are destroyed. Yeasts survive for 7 months to 3 years on or in fruit at low temperature. In foods, in general, yeasts survive 3–15 months.

Molds or fungi may be found in 42% of margarine samples. In or on vegetables they may live for 16 months but 90% are destroyed by the freezing process.

On fruit or in fruit juices at low temperature, viability of 7 months to 3 years has been demonstrated.

TABLE F1
The Survival of Bacillus Species in Food

Factor(s)	Survival	Reference	
MILK			
B. anthracis			
Milk from udder of cow that had died of the disease	10 yr.	Morris	1921
DAIRY PRODUCTS			
B. sp.			
Margarine	Recov. 72%	Foltz	1951
CEREAL			
B. anthracis			
Oats	Present	McFayden	1895

TABLE F1—(Continued)

The Survival of Bacillus Species in Food

Factor(s)	Survival	Reference	
VEGETABLES			
B. anthracis			
Roots of corn	50 d.	Beranek	1948
Plants, lima bean	Recov. 6–11 d.	Russell	1893
FRUITS			
B. mycoides			
Fruits, —5 F.	Resisted —5 and 15 F. better than higher temp.	Campbell	1932

TABLE F2

The Survival of Brucella Species in Food

Factor(s)	Survival	Reference	
MILK			
Br. abortus			
Cream, 8 C.	Bovine strain 8 d.	Carpenter	1928
Cream, 8 C.	Bovine and human strain 10 days	Carpenter	1928
Sour milk	5 d.	Honda	1938
Milk, home pasteurized	Inoc. 100,000/ml.; 5–10 min.	Huddleson	1949
Raw milk		Jones	1943
Raw cream	Recov.: 11 out of 13	Pullinger	1935
Sterilized and raw milk	Some after 50 min. recov.	Seelemann	1938
Milk, 145 F.	30 min.	Smith	1932
Milk, flash pasteurization	Org. survived	Smith	1932
Milk, 4–8 C., 18–19 C., 22–24 C.	Inoc. 0.5 cc milk, 1–7 d.	Stockmayer	1935
Milk +0.25% Boric acid, 4–8 C., 18–19 C., 22–24 C.	Inoc. 0.5 cc milk, 1–7 d.	Stockmayer	1935
Milk +0.5% Boric acid, 4–8 C., 18–19 C., 22–24 C.	Inoc. 0.5 cc milk, 1–7 d.	Stockmayer	1935
Milk +1% Boric acid, 4–8 C., 18–19 C., 22–24 C.	Inoc. 0.5 cc milk, 1–7 d.	Stockmayer	1935
Milk, +2% Boric acid, 4–8 C., 18–19 C., 22–24 C.	Inoc. 0.5 cc milk, 1–7 d.	Stockmayer	1935
Milk, R. T.	3–4 d.	Van Drimmelen	1948
Br. melitensis			
Sheep's milk, 16 C., pH 6.8–6.0	22–40 d.	Versilova	1937
Sheep's milk, 16 C., pH 4–5	30 d.	Versilova	1937
Milk, 37 C.	Few days	Versilova	1937
Br. suis			
Milk, 62 C., open coil pasteurizer	4 min.	Murray	1932
Milk foam in outlet, 63 C.	30 min.	Murray	1932

TABLE F2—(Continued)

The Survival of Brucella Species in Food

Factor(s)	Survival	Reference	
DAIRY PRODUCTS			
Br. abortus			
Butter, 8 C.	81–32 d.	Carpenter	1928
Butter, 46 F.	142 d.	Carpenter	1928
Cheese	60 d.	Daklberg	1946
Butter, 8 C.	Inoc. artificial; 32–142 d.	Fitch	1933
Ice cream	Present	Fitch	1933
Cheese	Few days	Pullinger	1935
Ice cream, 30 F.	1 mo.	Thompson	1933
Roquefort cheese	2 mo.	Thompson	1933
Cheese	2 mo.	Voille	1931
Ice cream, —23.2 C.	7 yr.	Wallace	1938
Butter and cheese	Few days	T. & W.	1946
Br. melitensis			
Goat cheese	Present	Eyre	
Cheese	Most common	Fabian	1947
Cheese of infected goat milk	6–20 d.	Peres	1936
Cheese from unpasteurized goat milk	38 d.–1 yr.	Stiles	1945
Brynza cheese, 11–14 C.	45 d.	Versilova	1937
Br. melitensis			
Ice cream, —23.2 C.	5 yr.	Wallace	1938
Ice cream	30 mo. +	Wallace	1938
Br. suis			
Ice cream, —23.2 C.	4 yr.	Wallace	1938
Ice cream	30 mo. +	Wallace	1938
Br. (spp.)			
Butter from infected cow, salted and unsalted	4 mo.	Bryan	1944
Cheese, 4.4 C.	Inoc. 1,000/ml.; >6 mo. <1 yr.	Gilman	1946
Cheddar cheese	Inoc. 700–800/ml.; >3 mo. <1 yr.	Gilman	1946
Cheddar cheese, 1.1–2.7 C.	>41 d.–84 d.	Gilman	1946
Cheese	Acidity decreases viability of org.	Lerche	1931
MEAT			
Br. melitensis			
Cured ham, at. infection, in ice box	21 d., none after smoking	Hutchings	1951
BEVERAGES			
Br. abortus			
Beer	3 d.	Serpa Santos	1939
Wines	1 hr.	Serpa Santos	1939

TABLE F3

The Survival of Clostridium Species in Food

Factor(s)	Survival	Reference	
DAIRY			
Cl. (spp.)			
Cheese	Most common	Fabian	1947
MEAT			
Cl. botulinum			
Cooked meat and fish	Spores present	Editorial	1926
Canned salmon,beef,sardines, clam juice, duck	Spores present	Editorial	1926
Cl. sporogenes			
Putrid meat, 50 C.	No growth in 9 d.	Haines	1944
Spore forming anaerobes			
Roast beef, canned in 1824	3 strains viable	Wilson	1938
Tripe, canned 1880	Not found	Wilson	1938
Cl. (spp.)			
Salted fish	.....	T. & W.	1946
Cooked meat and fish	.....	T. & W.	1946
VEGETABLE			
Cl. tetani			
Various vegetables	Present	Dubovsky	1922
Cl. botulinum			
Home canned string beans, corn, asparagus, spinach, pimento, pickles	Spores present	Editorial	1926
Vegetables grown in contaminated soil	Present	Parry	1946
Peas, string beans, corn, and spinach; 10–12 C., 19–21 C., and 24–26 C.	>3 mo.	Starin	1926
Peas, 10 F.	No toxin was produced when containers were defrosted and immediately examined or when stored in ice box 3 d.	Straka	1932
Peas, frozen, 42F., pH 5.6–6.5	Inoc. 100,000,000; Recov. 0; <7 d.	Straka	1935
Peas, frozen, 50 F.	Inoc.: conc.; Rev. 0; >7 d.	Straka	1935
Peas, frozen, 60 F., pH 4.6–6.7	>3 d.	Straka	1935
Peas, frozen, 60 F., pH 4.6–6.2	>6 d.	Straka	1935
Peas, frozen, 80 F., pH 4.3–6.6	Inoc.: conc.; >2 d.	Straka	1935
Vegetables, —16 C.	>2 yr.	Tanner	1931
Vegetables, —16 C.	14 mo.	Tanner	1931
Vegetables, 100 C.	90–80 min.	Weiss	1921
Vegetables, 105 C.	30–70 min.	Weiss	1921
Vegetables, 110 C.	10–20 min.	Weiss	1921
Spore forming anaerobes			
Carrots, canned 1885	Not found	Wilson	1938

TABLE F3—(Continued)

The Survival of Clostridium Species in Food

Factor(s)	Survival	Reference	
VEGETABLES, Continued			
Cl. (spp.)			
Cans of spinach	Inoc.: 800,000; 50% of the org. died in 18 hr.	Koser	1921
FRUIT			
Cl. sporogenes			
Dried fruit, 45 C.	Grown in 24 hr.	Haines	1944
Cl. (spp.)			
Fruit, —5 F.	140 d.	Campbell	1932
Fruit, —16 C.	>2 yr.	Tanner	1931
GENERAL FOOD			
Cl. botulinum			
Various foods, 35 C., artificially contaminated	Recov.: certain toxin; 1 yr.	Schoenholz	1923
Variety of foods, —16 C.	1 yr.	Wallace	1933
Acid foods, 100 C.	50 min.	Weiss	1921
Acid foods, 105 C.	30 min.	Weiss	1921
Acid foods, 110 C.	15 min.	Weiss	1921
Various types of food	.....	T. & W.	1946

TABLE F4

The Survival of Escherichia Coli in Food also Paracolobactrum and Aerobacter

Factor(s)	Survival	Reference	
MILK			
E. coli			
Cream, 30% butter fat, freezing at —15 C.	Reduction 61% in 3 hr.	Hilliard	1915
Milk dil., frozen	Less dil. the larger the survival	Keith	1913
Milk, —21 to —78 C.	Inoc. 100,000/ml., more resistant to freezing than thawing	Lund	.....
Milk, pH 4.2	Growth checked	Palladina	1935
Milk, plus 5% NaCl, pH 4.6	24 hr.	Palladina	1935
Skim milk or cream, 20 C.	Well	Robinton	1945
DAIRY PRODUCTS			
E. coli			
Milk curds	48–96 hr.	Bhat	1949
Cheese	12 mo.	Crossley	1942
Margarine	Isolated from 8%	Foltz	1951
Ice cream	In 90% of samples	Murgia	.....
Butter and margarine, rapid develop. of acidity and prompt salting	Check growth	Palladina	1935
Butter, 14 F.	8 wk.	Rice	1938
Butter, 60 F.	Did not mult. but survived long	Rice	1938

TABLE F4—(Continued)

The Survival of Escherichia Coli in Food also Paracolobactrum and Aerobacter

Factor(s)	Survival	Reference	
EGGS			
E. coli			
Frozen eggs	Recov. 50% of colonies examined were coliform	Colien	1942
Frozen eggs, —9 C.	14 mo.	Hartsell	1951
Egg whites, —15 C.	Recov. 0, 3 mo.	Johns	1946
Frozen eggs (white)	Recov. <10, 5 yr.	Schneiter	1943
Frozen eggs (yolk)	Recov. 4,000, 5 yr.	Schneiter	1943
MEAT			
E. coli			
Salt fish blocks, 5–6 C.	72 d.	Frank	1941
Frozen shrimp	In 60% of all samples	Holmes	1949
Fish and meat		Ignatovich	1935
Sausage, 6 d. drying	13 d.	Mueller-Claus	1938
Sausage, 35 C.	>24 hr.	Mueller-Claus	1938
VEGETABLES			
E. coli			
Veg., 20 C.	Recov. 60% out of 70 for 1 yr.	Burton	1949
Cantaloupes, —4 F.	>1 yr.	Burton	1949
Veg.	In 90% of samples	Murgia	.....
Tomatoes with bacteria sprayed on	5 min.	Rudolfs	1951
Mushrooms, —9.4 C.	6 mo.	Smart	1934
FRUITS			
E. coli			
Fruits	In 90% of samples	Maurgia	.....
Fruit in cold water	Recov. 100%, few min.	Spaini	1944
FRUITS, Continued			
E. coli			
Fruit in boiling water	Recov. 70% few min.	Spaini	1944
Cherries, —17.8 and —40 C.	2–3 mo.	Wallace	1933
Cherry juice, —17.8 and —40 C.	<4 mo.	Wallace	1933
Good oranges, 17.8 C., pH 3.64	Inoc. 12,300/cc, Recov. 2,800/cc, 7 mo.	Wolford	1948
Soft rotten oranges, 17.8 C. pH 3.74	Inoc. 31,500,000/cc, Recov. 1,250,000/cc, 8 mo.	Wolford	1948
BEVERAGES			
E. coli			
Beer	Contained many, from water to dilute beer	Buttiaux	1949

TABLE F4—(Continued)

The Survival of Escherichia Coli in Food also Paracolobactrum and Aerobacter

Factor(s)	Survival	Reference	
MILK			
A. aerogenes			
Milk, pH 4.2	Growth checked	Palladina	1935
Milk, 5% NaCl, pH 4.6	24 hr.	Palladina	1935
DAIRY PRODUCTS			
A. aerogenes			
Butter, 14 F.	8 wk.	Rice	1938
Salted butter, 60 F.	Did not mult., but survived long	Rice	1938
EGGS			
P. sp.			
Turkey and chicken egg albumin, incubated	Slight effect on bacteria	Gregory	1948
VEGETABLES			
A. aerogenes			
Cantaloupes, —4 F.	>1 yr.	Burton	1949
BEVERAGES			
A. sp.			
Beer	Contained 1000/ml.	Buttiaux	1949

TABLE F5

The Survival of Micrococcus Species in Food

Factor(s)	Survival	Reference	
MILK			
M. aureus			
Milk, 65–81 C.	Recov. 0, 1 d.	Lazarus	1890
Milk, —21 to —78 C.	Inoc. 100,000/ml., more resistant to freezing than thawing	Lund	.....
DAIRY PRODUCTS			
M. sp.			
Margarine	56%	Foltz	1951
EGGS			
M. aureus			
Egg powdered, R.T., stored in packet	Inoc. 2,000, Recov. 20, 70 d.	Haines	1944
Frozen eggs, —9 C.	12 mo.	Hartsell	1951
MEAT			
M. aureus			
Putrid meat, 44 C.	No growth	Haines	1944
Canned roast beef, 22–37 C.	Inoc. 30–5,000 org./gm., >60 d.	Surgella	1945

TABLE F5—(Continued)
The Survival of Micrococcus Species in Food

Factor(s)	Survival	Reference	
SAUCE			
M. spp.			
Salad dressing and mayonnaise	M. more resistant than S.	Wethington	1950
Mayonnaise, 37 C., pH 3.8, 0.48% acid	96 hr.	Wethington	1950
Salad dressing, 37 C., pH 3.2, 1.1% acid	30 hr.	Wethington	1950
Mayonnaise with egg yolk, 0.51% acid, pH 4.0, fresh	78 hr.	Wethington	1950
Mayonnaise with egg yolk, 0.51% acid, pH 4.0, emulsol	72 hr.	Wethington	1950
Salad dressing with egg yolk, 1.02% acid, pH 3.30, fresh	48 hr.	Wethington	1950
Salad dressing with egg yolk, 1.02% acid, pH 3.30, emulsol	16 hr.	Wethington	1950
Mayonnaise, pH 5.0, 0.15% acid	144 hr.	Wethington	1950
Salad dressing, pH 5.0, 0.15% acid	144 hr.	Wethington	1950
VEGETABLES			
M. aureus			
Plants, lima beans	Recov. 3, 13 d.	Russell	1893
M. sp.			
Asparagus, —17.8 C.	Inoc. 25.5%, Recov. 85.8%, 8 mo.	Lockhead	1938
Spinach, —17.8 C.	Inoc. 8.6%, Recov. 63.2%, 8 mo.	Lockhead	1938
Peas, —17.8 C.	Inoc. 21.3%, Recov. 44.4%, 8 mo.	Lockhead	1938
Beans, —17.8 C.	Inoc. 7.0%, Recov. 72.0%, 8 mo.	Lockhead	1938
Corn, —17.8 C.	Inoc. 20.7%, Recov. 78.7%. 8 mo.	Lockhead	1938
FRUITS			
M. aureus			
Dried fruit, 44 C.	No growth	Haines	1944
Sliced sweetened strawberries, —18 C.	Inoc. 500/gm., 6 mo.	McCleskey	1941
M. sp.			
Orange juice, —4 C.	50 hr.	Beard	1932

TABLE F6
The Survival of Microorganisms in Food

Factor(s)	Survival	Reference	
MILK			
Corynebacterium diphtheriae			
Cream, frozen	>4 d.	Bolten	1918
Milk	Present	Trevelyan	1898

TABLE F6—(Continued)
The Survival of Microorganisms in Food

Factor(s)	Survival	Reference	
MILK, Continued			
Lactobacillus casei			
Milk, —21 to —78 C.	Inoc. 100,000/ml., more resistant to freezing than thawing	Lund	.....
Lactobacillus acidophilus			
Milk, 116 C.	15 min.	Morrison	1930
Rickettsia spp.			
Ster. skim milk, 26–28 C.	Inoc. 0.5 ml. of 2x10–1 cotton rat liver, 24 hr.	Anderson	1944
Raw milk	Not given	Huebner	1948
Rickettsia			
Coxiella burneti			
Milk, R.T.	7 d.	Babudieri	1950
Milk, 37 C., air dried stored	30 d.	Babudieri	1950
DAIRY PRODUCTS			
Achromobacter delmarvae			
Butter	239 d.	Berry	1927
Butter and margarine, rapid develop. of acid and prompt salting	Growth checked	Palladina	1935
Bacterium linens			
Cheddar cheese, 10 C., pH 5.13	4 mo.	Albert	1944
Corynebacterium diphtheriae			
Butter	1 mo.	Minn. St. Bd. of Health	1911
Rickettsia			
Coxiella burneti			
Cheese made with infected milk	46 d.	Babudieri	1950
Butter, below freezing	41 d.	Jellison	1948
Lactobacillus sp.			
Butter	275–462 d.	Tanner	1944
MEAT			
Corynebacterium diphtheriae			
Sausage, 85 C. for 70 min.	24 hr.	Mueller-Claus	1938
Animal parasites			
Trichinella spiralis			
Pork, —15 C.	24–36 hr.	Tanner	1944
Trichina larvae			
Pork, —27 C.	Recov. 0, 36 hr.	Gould	1949
Pork, —30 C.	Recov. 0, 24 hr.	Gould	1949
Pork, —33 C.	Recov. 0, 10 hr.	Gould	1949
Pork, —35 C.	Recov. 0, 40 min.	Gould	1949
Pork, —37 C.	Recov. 0, 2 min.	Gould	1949
CEREAL			
Pasteurella tularensis			
Grain	Present	Ayres	1948
Grain contaminated with urine or feces of infected mice	Present	Zeiss	1943

TABLE F6—(Continued)

The Survival of Microorganisms in Food

Factor(s)	Survival	Reference	
VEGETABLES			
Lactobacillus cucumeris			
Peas, 15 F.	>2 yr.	Berry	1933
Lactobacillus spp.			
Veg., —10 C.	2 yr.	Berry	1933
Peas, —10 C.	2 yr.	Weiser	1951
Pseudomonas aeruginosa			
Plants	Recov. many, 69 d.	Russell	1893
Bacteria and parasites			
Veg., from irrigation water	Found	Wright	1950
FRUIT			
Proteus vulgaris			
Cherry juice, —17.8 C. and —40 C.	<4 wk.	Wallace	1933
GENERAL			
Pasteurella tularensis			
Food stuff	Present	Schuller	1943
Food, contaminated with urine or feces of infected mice	Present	Zeiss	1943

TABLE F7

The Survival of Microorganisms in Food (General)

Factor(s)	Survival	Reference	
MILK			
Milk, frozen and stored	Lowers no. of bacteria	Babcock	1947
Raw milk	Germicidal action decreases no.	Chambers	1920
Cream, —5 to —10 F.	Decreases during storage and freezing	Fabian	1943
Powdered skim milk, R.H. 5–20%	Inoc. 11,100/g. at 37 C. and 23,800/g. at 30 C., Recov. Max. survival, 48 wk.	Higginbottom	1948
Whole milk, 37 C., R.H. 10–20%	Inoc. 91,000/g., Reduct. 99.9%, 72 wk.	Higginbottom	1948
Milk used in coffe or tea	Present	Hill	1909
DAIRY PRODUCTS			
Margarine	42% had plate cts. of 100/ml. or less	Foltz	1951
Ice cream	Lower in winter mo.	Tanner	1944
EGGS			
Powdered egg, 60 C.	99% reduct. 1 d., decrease proportional to increase in temp.	Gibbons	1943

TABLE F7—(Continued)

The Survival of Microorganisms in Food (General)

Factor(s)	Survival	Reference	
EGGS (Continued)			
Egg whites, frozen	Low cts.	Verge	1928
Egg yolks, frozen	Low cts.	Verge	1928
Egg whites, R.T.	High cts.	Verge	1928
Egg yolks, R.T.	High cts.	Verge	1928
Eggs, 0 F.	Greater destruction of bacteria than at lower temp.	Winter	1947
MEAT			
Chicken-a-la-King	In ¼ samples of precooked food	Buchbinder	1949
Hamburger steak, unfrozen	Recov. >24,300/gm.	Geer	1933
Hamburger steak, frozen	Recov. >1,100,000/gm.	Geer	1933
Dehydrated meat, 15 C., in air, R.H. 4.5%	Inoc. 700,000, Recov. 9,400; 10 wk.	Haines	1944
Dehydrated meat, 15 C., in air, R.H. 2.0%	Inoc. 2,000,000, Recov. 37,000; 6 wk.	Haines	1944
Dehydrated meat, 15 C., in air	Inoc. 24,000,000; Recov. 29,300; 7 mo.	Haines	1944
Dehydrated meat, 15 C., in nitrogen	Inoc. 24,000,000; Recov. 669,000; 12 mo.	Haines	1944
Shrimp, —40 C.	Recov. greatly reduced in peeled, >12 mo.	Holmes	1949
Shrimp, —12 C.	More destructive than lower temp.	Holmes	1949
Meat	Present	Jensen	1945
Lamb chop (fat), —6.6 C.	Recov. 38,300, 6 wk.	Prescott	1932
Lamb chop (fat), —12 C.	Recov. 44,400, 6 wk.	Prescott	1932
Lamb chop (fat) —18 C.	Recov. 67,700, 6 wk.	Prescott	1932
Fish (haddocks), —4 C.	Start 47, Recov. 260, 7 wk.	Prescott	1932
Fish (haddocks), —6.6 C.	Start 47, Recov. 820 7 wk.	Prescott	1932
Fish (haddocks), —12 C.	Start 47, Recov.560, 7 wk.	Prescott	1932
Fish (haddocks), —18 C.	Start 47, Recov. 75	Prescott	1932
VEGETABLES			
Frozen veg., —18 C.	>4 yr.	Berry	1937
Vegetable tissue	Present	Galippe	1887
Dried veg., 65–80 C.	Present	Haines	1944
Frozen veg., 10, 0, —10 F.	Inoc. 50,000/g., Recov. no increase	Hucker	1951
Pickled veg.	Intestinal pathogens	Lin	1945
Vined peas, <40 F.	95% reduction	Link	1949
Frozen packed veg., —17.8 C.	Some present 9 mo.	Lockhead	1936
Peas, —20 C., brine packed	24 wk.	MacFarlane	1940
Spinach, —6.6 C.	Start 2170, Recov. 1190, 6 wk.	Prescott	1932
Spinach, —12 C.	Start 2170, Recov. 1350, 6 wk.	Prescott	1932
Spinach, —18 C.	Start 2170, Recov. 1350, 6 wk.	Prescott	1932

TABLE F7—(Continued)

The Survival of Microorganisms in Food (General)

Factor(s)	Survival	Reference	
VEGETABLES (Continued)			
Veg., bact. in soil	No results	Remlinger	1909
Peas and whole kernel corn frozen in liquid air or in air blast	Did not mult.	Van Eseltine	1948
Veg.	Low temp., moist soil. organic matter increase viability of pathogenes, and other pathogenes reduce survival	Rudolfs	1950
FRUITS			
Berries, airtight, frozen	Large decrease, greater decrease at high temp.	Berry	1933
Apple juice, —70 to —21 C.	Reduced 90–96% 1 mo.	Berry	1932
Apple juice, —5, —9.4, 6–7 C.	Survival <10% 1 mo.	Berry	1934
Frozen berries	Death more rapid at —9.40 than at —20.6 C.	Berry	1936
Cherries, washed in NaCl	Cts. 1200 to 600,000 present even after 3rd. washing	Hoder	1928
Blackberries		Hoder	1928
Currants		Hoder	1928
Yellow Plums		Hoder	1928
Pears		Hoder	1928
Damson plums		Hoder	1928
Fruits, moist	>15 d.	Mills	1925
Fruits, decayed portion	7–42 d.	Mills	1925
Cider, —10 C.	31 wk.	MacFarlane	1940
Raspberries, —20 C.	26 wk.	MacFarlane	1940
Strawberries, —6.6 C.	Start 1900, Recov. 280, 6 wk.	Prescott	1932
Strawberries, —12 C.	Start 1900, Recov. 960, 6 wk.	Prescott	1932
Strawberries, —18 C.	Start 1900, Recov. 2200, 6 wk.	Prescott	1932
Raspberries, —6.6 C.	Start 50,500, Recov. 275, 6 wk.	Prescott	1932
Raspberries, —12 C.	Start 50,500, Recov. 638, 6 wk.	Prescott	1932
Raspberries, —18 C.	Start 50,500, Recov. 1520, 6 wk.	Prescott	1932
Orange juice, —6.6 C.	Start 2410, Recov. 1100, 20 d.	Prescott	1932
Orange juice, —12 C.	Start 2410, Recov. 1090, 20 d.	Prescott	1932
Orange juice, —18 C.	Start 2410, Recov. 1160, 20 d.	Prescott	1932
Fresh strawberries, 15 F.	Inoc. fungi, yeast, bact., 1–3 yr.	Smart	1934
Strawberries in sealed tins	3 yr.	Smart	1934
Fruits, —9.4 C.	>4 yr.	Smart	1935
Blueberries, frozen	Inoc. before freezing >100,00/gm., Recov. <1%, 7 mo.	Smart	1937

TABLE F7—(Continued)

The Survival of Microorganisms in Food (General)

Factor(s)	Survival	Reference	
FRUITS (Continued)			
Blueberries, in 50% sugar syrup, —6.7 C.	99.9% reduct., 9 mo.	Smart	1939
Blueberries, in 50% sugar syrup, —17.8 C.	60% reduct., 9 mo.	Smart	1939
Blackberries, packed in 40% B. syrup	More recovered at —20 C. than —10 C.	Weiser	1951
CEREAL			
Soy beans, alfalfa	Viable 6–9 mo.	Fellers	1919
Pop corn, unpopped	Contained 100,000/g.	Breazeale	1951
Pop corn, popped	Contained 10/g.		
GENERAL			
Frozen foods	Fats and sugar soln. protect and acids destroy	James	1933
Food, —25.2 C.	>10 hr.	Tanner	1944
Food, pH 2	Practically all destroyed	Virtanen	1940

TABLE F8

The Survival of Mycobacterium Tuberculosis in Food

Factor(s)	Survival	Reference	
MILK			
Human			
Milk	10 d.	Heim	1889
Sour milk	4 wk.	Honda	1938
Milk, 58 C., pH 6.7, 6.3, 6.0	30 min.	Katrandjieff	1929
Milk, 60 C.–63 C., pH 6.7, 6.3, 6.0	30 min.	Katrandjieff	1929
Sour milk, 200 cc., R.T., pH 4–5	Inoc. 1 loop cult., viable 7 d.	Kliewe	1937
Cream, 60 C.–80 C.	2 min.	Mohler	.....
Milk, frozen, —8 C. to —20 C.	2 yr.	McCallum	1934
Ster. milk, 30 C.	Inoc. 500–2000/ml., 20 d.	Mattick	1946
Raw cream	Recov. 16 out of 31 samples	Pullinger	1935
Bovine			
Sour milk	10 d.	Honda	1938
Sour milk, 200 cc., R.T.	Inoc. 1 loop cult., viable 20 d.	Kliewe	1937
DAIRY PRODUCTS			
Human			
Butter, 15–22 C.	30 d.	Heim	1889
Curds, 15–22 C.	2 d.	Heim	1889
Whey, 15–22 C.	14 d.	Heim	1889
Cheese, 15–22 C.	14 d.	Heim	1889

TABLE F8—(Continued)

The Survival of Salmonella Species in Food

Factor(s)	Survival	Reference	
DAIRY PRODUCTS (Continued)			
Cheese	2 mo. (summer)	Kankaapaa	
Cheese	140 d.	Kastli	1948
Emmenthal and Gruyere cheese	Inoc. >1/cc., 20–30 d.	Kastli	1949
Munster and Camembert cheese	Inoc. >1/cc., 47 d.	Kastli	1949
Tilsit cheese	Inoc. >1/cc., 232 d.	Kastli	1949
Cheese with low fat milk	Inoc. >1/cc., 326 d.	Kastli	1949
White cheese	>14 d.	Loncin	1950
Butter of milk held at 145 F. for 30 min.	Present	Smith	1932
Ice cream	6½ yr.	Wallace	1938
Ice cream	30 mo.	Wallace	1933
Butter from t.b. milk, pasteurized at 55, 60, and 70 C.	Survived pasteurization	Cookson	1926
Bovine			
Ice cream	6½ yr.	Wallace	1938
Ice cream	30 mo.	Wallace	1933
Avium			
Ice cream	4½ yr.	Wallace	1938
Ice cream	30 mo.	Wallace	1933

TABLE F9

The Survival of Salmonella Species in Food

Factor(s)	Survival	Reference	
MILK			
S. paratyphi A			
Milk, ice box	170 d.	Barry	1927
Milk, 37 C., 1 lactic acid to 250 milk	Inoc. 1 loop of 24 hr. cult; Recov. 0; 72–60 hr.	Kaiser	1921
Raw milk, 18 C., pH 5.04–4.84	11 d.	Kliewe	1935
S. paratyphi B			
Milk, 37 C., 1 lactic acid to 250 milk	Inoc. 1 loop of 24 hr. cult; Recov. 0; 12–60 hr.	Kaiser	1921
Milk, 37 C., 1 lactic acid to 500 milk	Inoc. 1 loop of 24 hr. cult; Recov. 0; 72 hr.	Kaiser	1921
Milk, 37 C., 1 lactic acid to 1200 milk	Inoc. 1 loop of 24 hr. cult; Recov. 0; 60 hr.	Kaiser	1921
Milk, ice box temp.	324 d.	Berry	1927
Raw milk, 18 C., pH 5.04–4.84	11 d.	Kliewe	1935
Milk, pH 4.2	Growth checked	Palladina	1935
Milk, 5% NaCl, pH 4.6	24 hr.	Palladina	1935
Milk, pH 4.7–5.1	63 d.	Wilson	1945
S. paratyphi			
Ster. milk, 17–20% acid, 37 C.	Recov. 0; 1–2 wk.	Kliewe	1935
Ster. milk, 24.13%, 20 C.	Viable 29 d.	Kliewe	1935
Ster. milk, 18.50%, 37 C.	Recov.0; 9 d.	Kliewe	1935

TABLE F9—(Continued)

The Survival of Salmonella Species in Food

Factor(s)	Survival	Reference	
MILK, Continued			
Ster. milk, 2.6–15.0%, 20 C.	Recov. 0; 14 d.	Kliewe	1935
Ster. milk, 20.6%, 8 C.	Recov. 0; 36 d.	Kliewe	1935
Ster. milk, 33%, 20 C.	Viable 4–5 wks.	Kliewe	1935
Ster. milk, 26%, 8 C.	6–7 wk.	Kliewe	1935
Raw milk, 27.04%, 37 C.	Recov. 0; 14 d.	Kliewe	1935
Raw milk, 33.25%, 37 C.	Recov. 0; 4 d.	Kliewe	1935
Raw milk, 27.36–18.3%, 37 C.	Recov. 0; 12 d.	Kliewe	1935
Raw milk, 31.51%, 20 C.	Recov. 0; 13 d.	Kliewe	1935
Raw milk, 17.82%, 20 C.	Recov. 0; 7 d.	Kliewe	1935
Raw milk, 20.15%, 20 C.	Recov. 0; 3 d.	Kliewe	1935
Raw milk, 22.61%, 20 C.	Recov. 0; 7 d.	Kliewe	1935
Raw milk, 22.80%, 8 C.	Recov. 0; 14 d.	Kliewe	1935
Sour milk, pH 5.04–4.84	11 d.	Kliewe	1935
Milk, R.T., acidity .7	4 d.	Marsh	1918
Milk, suspn, 63 C., in an open reagent glass	15 min.	Orskov	1925
Milk, suspn, 63 C., in water bath in glass with rubber stopper	Recov. 0; 3 min.	Orskov	1925
Milk suspn, 63 C., warmed at 48 C. until a 1.5 cm. ring of dried milk forms	Recov. 0; 50 min.	Orskov	1925
Milk, pH 4.8	63 d.	Wilson	1945
S. enteritidis			
Milk, ice box temp.	180 d.	Berry	1927
Evapor. milk; 37 C., 6–9 C., 20 C.; pH 5.2, 7.2, 5.4	7 d.	Koser	1922
S. typhimurium			
Ster. milk, 22.28%, 20 C.	Viable 32 d.	Kliewe	1935
Ster. milk, 16.50%, 37 C.	Recov. 0; 13 d.	Kliewe	1935
Ster. milk, 24.13%, 8 C.	Recov. 0; 47 d.	Kliewe	1935
Ster. milk, 33%, 20 C.	Viable 4–5 wk.	Kliewe	1935
Ster. milk, 25%, 8 C.	6–7 wk.	Kliewe	1935
Raw milk, 12.15%, 37 C.	Recov. 0; 14 d.	Kliewe	1935
Raw milk, 33.20%, 37 C.	Recov. 0; 4 d.	Kliewe	1935
Raw milk, 25.77%, 37 C.	Recov. 0; 13 d.	Kliewe	1935
Raw milk, 35.22%, 20 C.	Recov. 0; 13 d.	Kliewe	1935
Raw milk, 25.05%, 20 C.	Recov. 0; 10 d.	Kliewe	1935
Raw milk, 25.54%, 20 C.	Recov. 0; 6 d.	Kliewe	1935
Milk, pH 4.2	Growth checked	Palladina	1935
Milk, 5%NaCl, pH 4.6	24 hr.	Palladina	1935
Sour milk, 9.75–13% acid, 8 C.	Recov. 0; 11 d.	Kliewe	1935
S. typhosa			
Raw milk, 0.27% acid	5 d.	Bassenge	1903
Raw milk, 0.36% acid	6 d.	Bassenge	1903
Raw milk, 0.63% acid	24 hr.	Bassenge	1903
Milk	Recov. 0; 2 hr.	Belin	1933
Milk, ice box and R.T.	(Miss.) 290 d. and 187 d.	Berry	1927
Sour milk, 1 d. old	Inoc. 100 cc.+cc.⅛ typhoid 1 mo.	Bolley	1898
Sour milk, 2 d. old	Inoc. 15 cc.+5 cc.; 5 d.	Bolley	1898

TABLE F9—(Continued)
The Survival of Salmonella Species in Food

Factor(s)	Survival	Reference	
MILK (Continued)			
S. typhosa			
Sour skim milk	Inoc. 100 cc.+½ cc. cult.; 5 d.	Bolley	1898
Sweet milk	Inoc. 100 cc.+ 24 hr. cult.; 1 mo.	Bolley	1898
Past. milk	Inoc. 100 cc., ⅛ cc. cult.; 10 d.	Bolley	1898
Whole ster. milk	Inoc. loop; 1 mo.	Bolley	1898
Sweet cream	Inoc. 200 cc., 8 cc. cult; 4 mo.	Bolley	1898
Milk ster. by discount past., loop inoc.	Loop inoc.; 4 mo.	Bolley	1898
Milk, 56 C. for 20 min.	Loop inoc.; 4 mo.	Bolley	1898
Fresh milk drawn in ster. tube	Loop inoc.; 3 mo.	Bolley	1898
Cream, freezing	Inoc. 5 cc. of cult.; >4 d.	Bolten	1918
Sour cream strongly acid	Inoc. 1 loop cult.; 10 d.	Bruck	1903
Milk	Inoc. 1 g.; several days	Cautley	1897
Sour milk, R.T., 2.25% acid	6–8 d.	Demme	1925
Milk, 13–18 C.	Viable <48 d	Heim	1889
Ster. milk	4 mo.	Hesse	1889
Milk, alternate freezing	Reduction <93–99%	Hillard	1918
Ster. milk, 17–20% acid, 37 C.	Recov. 0; 4 hr.	Kliewe	1935
Ster. milk, 30% acid, 8–20 C.	Recov. 0; several wk.	Kliewe	1935
Ster. milk, 25 min. in autocl., 5.25% acid	Viable 13 d.	Kliewe	1935
Ster. milk, 8.80–7.50% acid, 20 C.	Viable 14 d.	Kliewe	1935
Ster. milk, 23.88%, 20 C.	Viable 28 d.	Kliewe	1935
Ster. milk, 23–20.5%, 37 C.	Recov. 0; 24 hr.	Kliewe	1935
Ster. milk, 25.85% acid, 8 C.	Recov. 0, 44 d.	Kliewe	1935
Ster. milk, 33% acid, 20 C.	Viable 2 wk.	Kliewe	1935
Ster. milk, 26% acid, 8 C.	Recov. 0, 6 wk.	Kliewe	1935
Raw milk, 37 C	Recov. 0, 3 d.	Kliewe	1935
Raw milk, 37 C., 14.97% acid	Recov. 0, 12 d.	Kliewe	1935
Raw milk, 37 C., 33.73% acid	Recov. 0, 4 d.	Kliewe	1935
Raw milk, 37 C., 17.81% acid	Recov. 0, 11 d.	Kliewe	1935
Raw milk, 20 C., 38.25% acid	Recov. 0, 12 d.	Kliewe	1935
Raw milk, 20 C., 31.51% acid	Recov. 0, 6 d.	Kliewe	1935
Raw milk, 20 C., 24.45% acid	Recov. 0, 3 d.	Kliewe	1935
Sour raw milk, 20 C., 9.75–16.50% acid	Recov. 0, 3 d.	Kliewe	1935
Raw milk, 8 C., 20.38–22.35% acid	Recov. 0, 12 d.	Kliewe	1935
Raw milk, 8 C., 18.75–20.90% acid	Recov. 0, 14 d.	Kliewe	1935
Raw milk, 18 C., pH 7.17–6.94	Inoc. 2 loops, Recov. 0, 48 hr.	Kliewe	1935
Raw milk, 37 C., pH 5.02–3.56	Inoc. 2 loops, Recov. 0, 24 hr.	Kliewe	1935
Raw milk, 1 loop B. coli, 18 C., pH 3.94–7.93	Inoc. 1 loop, Recov. 0, 12 d.	Kliewe	1935
Raw milk, 37 C.	3 d.	Kredba	1935

TABLE F9—(Continued)

The Survival of Salmonella Species in Food

Factor(s)	Survival	Reference	
MILK, Continued			
Sour cream, R.T.	Inoc. 7 million, Recov. 0, 48 hr.	Krumwiede	1914
Cream, soured overnight in sterilizer	Inoc. 7 million, Recov. 0, 120 hr.	Krumwiede	1914
Cream, R.T.	Inoc. 375,000, Recov. 0, 96 hr.	Krumwiede	1914
Milk, 66 C.–74 C.	Recov. 0, 1 d.	Lazarus	1890
Milk, R.T., acid .7	5 d.	Marsh	1918
Fresh milk, acid 19–1.4	1–2 d.	Marsh	1918
Sour milk	3 mo.	Osler	1901
Milk, pH 4.2	Growth was checked	Palladina	1935
Fresh milk, 7–10 C.	Inoc. suspn. of agar, viable 11–13 d.	Pfuhl	1902
Milk, 3 C.	2 d.	Seitz	1886
Milk, 0.71% acid	8 d.	Wade	1928
Milk, 0.53% acid	30 d.	Wade	1928
Milk with S. lacticus, 0.84% acid, R.T., and 0.65%, ice box temp.	5 d. and 27 d.	Wade	1928
Milk, with diplococcus "x", 0.88% acid, ice box	86 d.	Wade	1928
Milk, with diplococcus "x", 1.04% acid, R.T.	34 d.	Wade	1928
Milk, 0.90% acid	1 d.	Wade	1928
Milk, 0.74, 0.78, 0.81%	4 d.	Wade	1928
Milk	20 d.	Washburn	1908
Milk, pH 4.9–5.1	63 d. plus	Wilson	1945
DAIRY PRODUCTS			
S. paratyphi A			
Butter	117 d.	Berry	1927
S. paratyphi B			
Butter	212 d.	Berry	1927
Milk curds	48–96 hr.	Bhat	1949
Yoghurt, 1.87% acid	Inoc. 1 loop 24 hr. cult. Recov. 0, 48–108 hr.	Kaiser	1921
Yoghurt, 0.29–0.82% acid	Inoc. 1 loop 24 hr. cult. Recov. 0, 12 hr.	Kaiser	1921
Yoghurt, 1.89–2.14% acid	Inoc. 1 loop 24 hr. cult. Recov. 0, 60–72 hr.	Kaiser	1921
Yoghurt, 2.35% acid	Inoc. 1 loop 24 hr. cult. Recov. 0, 120 hr.	Kaiser	1921
Yoghurt, 1.07% acid	Inoc. 1 loop 24 hr. cult. Recov. 0, 24 hr.	Kaiser	1921
Butter and margarine, rapid develop. of acid and prompt salting	Growth checked	Palladina	1935
Buttermilk	15 d.	Stever	1941
S. paratyphi types			
Milk curds	48–96 hr.	Bhat	1949
Butter (English), 15 C.	<112 d.	Pullinger	1938
Butter (English), 3 C.	>112 d.	Pullinger	1938
Buttermilk	15 d.	Stever	1941

TABLE F9—(Continued)

The Survival of Salmonella Species in Food

Factor(s)	Survival	Reference	
DAIRY PRODUCTS (Continued)			
Cheese	Most common	Fabian	1947
S. sp. (Type Newport)			
Butter, contamined by cooling water	Present	Eriksson	1941
S. enteritidis			
Butter	228 d.	Berry	1927
Milk curds	48–96 hr.	Bhat	1949
Butter (English), 15 C.	<112 d.	Pullinger	1938
Butter (English), 3 C.	>112 d.	Pullinger	1938
Ice cream, —23.2 C.	7 yr.	Wallace	1938
Ice cream, freezing	30 mo. +	Wallace	1933
S. typhimurium			
Butter	239 d.	Berry	1927
Ice cream	98 d.	Glass	1946
Butter and margarine, rapid develop. of acid and prompt salting	Growth checked	Palladina	1935
Cheese, 43–48 F.	Inoc. colby cheese, 302 d.	Tucker	1946
Ice cream, —23.2 C.	6 yr.	Wallace	1938
Ice cream, freezing	30 mo. +	Wallace	1938
S. choleraesuis			
Butter	49 d.	Berry	1927
S. typhosa			
Buttermilk, strongly acid	Inoc. 1 loop cult., 10 d.	Bruck	1903
Buttermilk, 22 C.	Inoc. 2 loop 24 hr. cult., 3 d.	Fraenkel	1898
Buttermilk, 37 C.	Inoc. 2 loop 24 hr. cult., 24 hr.	Fraenkel	1898
Buttermilk, pH 3.5–4.4	2–3 d.	Marsh	1918
Buttermilk, pH 3.1–4.5	1 d.	Marsh	1918
Buttermilk, R.T.	3 d.	Rubenstein	1902
Buttermilk, incubator	24 hr.	Rubenstein	1902
Cheese infected by water	6–10 mo.	Anon.	1944
Butter	22 d.	Berry	1927
Butter	110 d.	Berry	1927
Milk curds	48–96 hr.	Bhat	1949
Fresh creamery butter	Inoc. spots, 5 d.	Bolley	1898
Fresh creamery butter	Inoc. germs mixed, 5 d.	Bolley	1898
Fresh creamery butter	Inoc. 1 loop, 5 d.	Bolley	1898
Fresh creamery butter, salt 4 oz./lb.	Inoc. 1 loop, 5 d.	Bolley	1898
Unsalted churned butter	Inoc. 200 cc. & 8 cc. cult., 10 d.	Bolley	1898
Salted churned butter, 10 oz./lb.	Inoc. 200 cc. & 8 cc. cult., 10 d.	Bolley	1898
Fresh creamery butter, kept in cold storage	None found	Bolley	1898
Fresh creamery butter, salted in cold storage	None found	Bolley	1898
Cheese	80 d.	Bowman	1942
Butter, strongly acid	Inoc. 1 loop cult., 27 d.	Bruck	1903

TABLE F9—(Continued)

The Survival of Salmonella Species in Food

Factor(s)	Survival	Reference	
DAIRY PRODUCTS (Continued)			
S. typhosa			
Cheese, 58–60 F.	3 mo.	Campbell	1944
Cheese, 40–42 F.	6–10 mo.	Campbell	1944
Cheese, cheddar	<3 mo.	Foley	1945
Butter, 13–18 C.	Viable 21 d.–1 mo.	Heim	1889
Cheese, 35 C., alkaline	Viable 3 d.	Heim	1889
White cheese, 35 C.	Viable <24 hr.	Heim	1889
Curds, 35 C.	Viable 1 d.	Heim	1889
Whey	Viable 1 d.	Heim	1889
Cheese	4 wk.	Hesse	1889
Butter, weakly acid	4–5 d.	Laser	1891
Butter	6 d.	Lafar	
Cheese, 60 F., artifically infected	34–36 d.	Meyer	1944
Ice cream, frozen	Inoc. 70,000/cc., Recov. 450,000/cc. in 24 hr.	Mitchell	1915
Ice cream, frozen Knox gelatin added	Inoc. 400,000/cc., Recov. 60,000/cc. in 24 hr.	Mitchell	1915
Butter made from infected cream	Several days	Osler	1901
Butter and margarine, rapid develop. of acid and prompt salting	Growth checked	Palladina	1935
Fresh butter, 7–10 C.	Inoc. agar cult. ground in agar mortar, 24 d.	Pfuhl	1902
Gervais cheese	Inoc. agar cult., 24 d.	Pfuhl	1902
Ice cream	>2 yr.	Prucha	1926
All classes of butter	At least 80 d.	Pullinger	1938
Sweet and sour curds, pH 4.2–4.7	Inoc. 4 drops cult., Recov. 0, <1 hr.	Panja	1945
Cheese, R.T.	26 d.	Ranta	1941
Cheese, ice box	<75 d.	Ranta	1941
Ice cream, —19 C.	1 yr.	Tanner	1928
Colby cheese	45 d.	Thomasson	1944
Cheddar cheese, from contaminated milk, 1.04% acidity	36 d.	Wade	1928
Cheddar cheese (commercial)	63 d.	Wade	1928
Cheese, 1.12% acid, exposed to air	16 d.	Wade	1928
Cheese, 0.94% acid, exposed to air	13 d.	Wade	1928
Cheese, 0.97% acid, exposed to air	13 d.	Wade	1928
Cheese, 0.98% acid, no air	7 d.	Wade	1928
EGGS			
S. typhimurium			
Defrosted whole egg; –1, –9, and –18 C.	11 mo. +	Hartsell	1950
S. typhosa			
Defrosted whole egg	11 mo. +	Hartsell	1950
S. sp. (Type Oranienburg)			
Defrosted whole egg; –1, –9, and –18 C.	11 mo. +	Hartsell	1950

TABLE F9—(Continued)

The Survival of Salmonella Species in Food

Factor(s)	Survival	Reference	
EGGS (Continued)			
S. spp.			
Fermented albumen, R.T., dried	Recov. 100%	Ayres	1949
Fermented albumen, 120 F., dried	20 d.	Ayres	1949
Eggs	0.6% of samples	Felsenfield	1950
Eggs powdered	3% of samples	Felsenfield	1950
Spray dried eggs	Recov. in 9.9% of samples	Med.Res.Coun.	1947
Duck eggs	Present	Mallam	1946
Powdered egg, 35 F.	65 wk.	Wilson	1948
S. pullorum			
Raw egg	Inoc. feces, present	Mitchell	1946
MEAT			
S. paratyphi B			
Chicken chow mein, –25.5 C.	Inoc. 230×10^5/gm., Recov. 19×10^5/gm., 270 d.	Gunderson	1948
S. partyphi types			
Livers, brains, hamburger steak, fresh pork, sausage, pork and beef loaf, kidney, cooked pork, smoked sausage cured ham and bacon, beef, lamb, calf sweetbreads, and chicken livers	Present	Cherry	1942
Beef	1 of sample	Felsenfield	1950
Birds (inspected)	0.9% of samples	Felsenfield	1950
Birds (uninspected)	10.8% of samples	Felsenfield	1950
Pork (inspected)	14.3% of samples	Felsenfield	1950
Pork (uninspected)	26.8% of samples	Felsenfield	1950
Hamburger	17.6% of samples	Felsenfield	1950
S. enteritidis			
Corned beef	Inoc. 30–5000/gm., Recov. 30×10^6, >60 d.	Surgella	1945
S. typhimurium			
Chicken chow mein, –25.5 C.	Inoc. 167×10^5/gm., Recov. 34×10^5/gm., 270 d.	Gunderson	1948
S. anatum			
Chicken chow mein, –25.5 C.	Inoc. 100×10^5/gm., Recov. 4.2×10^5/gm., 270 d.	Gunderson	1948
S gallinarum			
Chicken chow mein, –25.5 C.	Inoc. 68.5×10^5/gm., Recov. 4.8×10^5/gm., 270 d.	Gunderson	1948
S. sp. (Type Newington)			
Chicken chow mein, –25.5 C.	Inoc. 75.5×10^5/gm., Recov. 2.2×10^5/gm., 270 d.	Gunderson	1948

TABLE F9—(Continued)

The Survival of Salmonella Species in Food

Factor(s)	Survival	Reference	
MEAT (Continued)			
S. typhosa			
Souse meat	3 mo.	Duff	1942
Salt fish blocks, 5–6 C.	22 d.	Frank	1941
Chicken chow mein, –25.5 C.	Inoc. 12.8×10^5/gm., Recov. 4.8×10^5/gm., 270 d.	Gunderson	1948
Oysters	10 d.	Herdman	1899
Shell oysters, 5–8 C., floated 1 hr. in sea water to which typhoid is added	3 strains 21–24 d.	Jordan	1925
Oysters, 10 C. and –2.8 to 14.4 C.	15 d.	Kinyoun	1925
Oysters, wet	Inoc. 160,000,000, Recov. 320, 4 d.	Klein	1905
Oysters, dry	Inoc. 160,000,000, Recov. 1220, 7 d.	Klein	1905
Oysters, wet, infected water	Inoc. 744,000/cc.; Recov. 44; 6 d.	Klein	1905
Oysters, dry, infected by water	Inoc. 744,000/cc.; Recov. 90; 9 d.	Klein	1905
Oysters, sterile sea water, wet	Inoc. 2,250,000; Recov. 105; 3 d.	Klein	1905
Oysters, sterile sea water, dry	Inoc. 2,250,000; Recov. 714; 4 d.	Klein	1905
Cockles in sea water	Inoc. 4 million/cc.	Klein	1905
Mussels in sea water	Inoc. 5,170,000/cc.	Klein	1905
Oysters	7–11 d.	Klein	1905
Sausage, 4 d. drying	13 d.	Mueller-Claus	1938
Sausage, 35 C.	24 hr.	Maurel	1910
Tuna, –3 to 22.5 C.	Inoc. 3371 g., Recov. 0, 39 d.	Tetsumoto	1930
Mackerel, –3 to 22.5 C.	Inoc. 3435 g., Recov. 0, 38 d.	Tetsumoto	1930
Cuttle fish, –3 to 22.5 C.	Inoc. 3399 g.. Recov. 0, 40 d.	Tetsumoto	1930
Oyster, –3 to 22.5 C.	Inoc. 3262 g., Recov. 0, 45 d.	Tetsumoto	1930
Sea cucumber, –3 to 22.5 C.	Inoc. 3413 g., Recov. 0, 31 d.	Tetsumoto	1930
Tuna, 22.5–31 C.	Inoc. 3498 g., Recov. 0, 22 d.	Tetsumoto	1930
Mackerel, 22.5–31 C.	Inoc. 3391 g., Recov. 0, 20 d.	Tetsumoto	1930
Cuttle fish, 22.5–31 C.	Inoc. 3444 g., Recov. 0, 22 d.	Tetsumoto	1930
Oyster, 22.5–31 C.	Inoc. 3365 g., Recov. 0, 24 d.	Tetsumoto	1930
Sea cucumber, 22.5–31 C.	Inoc. 3415 g., Recov. 0, 20 d.	Tetsumoto	1930
Shucked oysters, 98 F.	Inoc. 74,000,000/cc.; Recov. 0, 1 d.	Tonney	1925
Shucked oysters, 70 F.	Inoc. 74,000,000/cc.; Recov. 0, 4 d.	Tonney	1925

TABLE F9—(Continued)
The Survival of Salmonella Species in Food

Factor(s)	Survival	Reference	
MEAT (Continued)			
S. typhosa			
Shucked oysters, 45 F.	Inoc. 74,000,000/cc.; Recov. 0, 22 d.	Tonney	1925
Shell fish	.....	T & W	1946
SAUCES			
S. spp.			
Salad dressing, 37 C., pH 3.8, 0.48% acid	12 hr.	Wethington	1950
Mayonnaise, 37 C., pH 3.2, 1.10% acid	6 hr.	Wethington	1950
Mayonnaise, with egg yolk, 0.51% acid, pH 4.0 fresh	18 hr.	Wethington	1950
Mayonnaise, with egg yolk, 0.51% acid, pH 4.0, emulsol	12 hr.	Wethington	1950
Salad dressing, with egg yolk, 1.20% acid, pH 3.30, fresh	8 hr.	Wethington	1950
Salad dressing, with egg yolk, 1.20% acid, pH 3.30, emulsol	1 hr.	Wethington	1950
Salad dressing, pH 4.4, 0.4% acid	144 hr.	Wethington	1950
Mayonnaise, pH 5.0, 0.15% acid	144 hr.	Wethington	1950
CEREAL			
S. sp.			
Crust of rye bread, R.T.	6 mo.	Bachmann	1943
S. typhosa			
Bread, after baking, R.T.	3 hr. (same results when smeared with fecal suspn.)	Alves	1935
Crust of rye bread, R.T.	4½ mo.	Bachmann	1943
VEGETABLES			
S. enteritidis			
Corn and spinach	3 yr.	Doyle	1930
Canned peas	200 d.	Doyle	1930
Spinach, 20 C. and 6–9 C.	7 d.	Koser	1922
String beans, 20 C. and 6–9 C.	7 d.	Koser	1922
Corn, pH 6.2–6.0, 20 C. and 6–9 C.	7 d.	Koser	1922
Peas, pH 5.8–6.0, 20 C. and 6–9 C. and 37 C.	7 d.	Koser	1922
S. typhimurium			
Canned spinach	3 yr.	Doyle	1930
Corn, R.T.	Inoc. 700,000/cc.; 100 d.	Doyle	1930
Canned peas	200 d.	Doyle	1930
Green veg., R.T.	3–7 wk.	Felsenfield	1945
Green veg., refrigerator	5–11 wk.	Felsenfield	1945
Peas, -9 C., sharp frozen	Inoc. 14.55; Recov. 0, 9 wk.	Hartsell	1951
S. pullorum			
Green veg., R.T.	4–8 wk.	Felsenfield	1945
Onions	Not considerable time	Felsenfield	1945

TABLE F9—(Continued)

The Survival of Salmonella Species in Food

Factor(s)	Survival	Reference	
VEGETABLES (Continued)			
S. sp. (Type Oranienburg)			
Green veg., R.T.	2–7 wk.	Felsenfield	1945
Green veg., refrigerator	5–10 wk.	Felsenfield	1945
Peas, –9 C., sharp frozen at –25 C.	Inoc. 28.45x, Recov. 0, 0.273x, 12 wk.	Hartsell	1951
S. sp. (Type Montevidea)			
Green veg., refrigerator	5–10 wk.	Felsenfield	1945
Green veg., R.T.	2–7 wk.	Felsenfield	1945
S. typhosa			
Green washed herbs	Several days	Anon.	1923
Lettuce salad, washed at 1–6–12 hr. after infected with typhoid	As many at 12 hr. as at one	Ceredi	1929
Green veg., R.T.	25 d.	Creel	1912
Green veg., exposed to rain and sun part of day	31 d.	Creel	1912
Green veg., exposed all day	10 d.	Creel	1912
Peas, –9 C., sharp frozen at –25 C.	Inoc. 33.4×10^6; Recov. 0.124×10^6; 12 wk.	Hartsell	1951
S. typhosa			
Mushrooms	4–5 wk.	Hesse	1889
Plants	3–5 d.	Lominsky	1890
Radishes, grown in contaminated soil	>37 d.	Melick	1917
Lettuce, grown in contaminated soil	>21 d.	Melick	1917
Cabbage, 80 C., water	10 sec.	Ommyoji	1931
Carrots, 80 C., shaken in water	5 sec.	Ommyoji	1931
Soy bean sauce, pH acid, 25–17 C.	Inoc. 1 loop, >24 hr.	Wang	1945
Soy bean sauce, pH acid, 36–38 C.	Inoc. 1 loop, <24 hr.	Wang	1945
FRUITS			
S. paratyphi B			
Watermelon	120 hr.	Bhat	1948
Sliced sweet strawberries –18 C.	1 mo.	McCleskey	1941
Cherries, –17.8 and –40 C.	2–3 mo.	Wallace	1933
Cherry juice, –17.8 and –40 C.	4 wk.	Wallace	1933
S. paratyphi types			
Orange juice, –4 C.	170 hr.	Beard	1932
Sliced sweet strawberries –18 C.	Not recovered	McCleskey	1941
Orange juice, –4 C., pH 3.5	96 hr.	Beard	1932
S. enteritidis			
Tomatoes, 20 C. and 6–9 C.	7 d.	Koser	1922
S. typhimurium			
Sliced sweet strawberries –18 C.	Inoc. 500/gm., 6 mo.	McCleskey	1941
Cherries, –17.8 and –40 C.	2–3 mo.	Wallace	1933
Cherry juice, –17.8 and –40 C.	4 wk.	Wallace	1933
S. typhosa			
Sliced sweet strawberries, –18 C.	Inoc. 500/gm., 6 mo.	McCleskey	1941
Uncut sweet strawberries	14 mo.	McCleskey	1941

TABLE F9—(Continued)

The Survival of Salmonella Species in Food

Factor(s)	Survival	Reference	
FRUITS (Continued)			
S. typhosa			
Pears, >80 C., water	>1 min.	Ommyoji	1931
Surface of dates	68 d.	Smeall	1932
Cherry juice, –14 C.	Inoc. artificial, 2 wk.	Tanner	1931
Cherry juice, –16 C.	Inoc. artificial, 5 mo.	Tanner	1931
Cherry juice, –17.8 and –40 C.	<4 wk.	Wallace	1933
Cherries, –17.8 and –40 C.	2–3 mo.	Wallace	1933
Orange juice, –4 C., pH 3.5	170 hr.	Beard	1932
BEVERAGES			
S. typhosa			
Beer, 22–37 C.	1–3 d.	Humpesch	1949
Beer, 5 C.	38 d.	Humpesch	1949
Beer, with paratyphoid	5–10 wk.	Humpesch	1949
Beer, 4.86% alcohol, 1.79% acid	4 d.	SerpaSantos	1939
Wines	1 hr.	SerpaSantos	1939
S. spp.			
Beer, 22–37 C., with paratyphoid	1–3 d.	Humpesch	1949
Beer, natural with paratyphoid	5–10 wk.	Humpesch	1949
Carbon dioxide drinks	Effect of CO_2 greatest at 19–23 C.	Koser	1922
Red wine, 15 C., 4.90% sulphuric acid	Inoc. 2 drops, 2 hr.	Sabrazes	1907
White wine, 17–18 C., 5% acid	Inoc. 2 drops, 1 hr.	Sabrazes	1907
Cerons, 11–18 C.	Inoc. 2 drops, 5 min.	Sabrazes	1907
GENERAL			
S. sp.			
Anaerobic sludge used for fertilizer	7 d.	Wolman	1924

TABLE F10

The Survival of Shigella Species in Food

Factor(s)	Survival	Reference	
MILK			
Sh. dysenteriae			
Fresh milk, 7–10 C.	18–27 d.	Pfuhl	1902
Milk, pH 4.8–4.9	53 d.	Wilson	1945
Sh. paradysenteriae (Flexner)			
Milk, pH 4.5–4.7	53 d.	Wilson	1945
Sh. paradysenteriae			
Sterile milk, 17–20 C.	Until it dries up	Frost	1905
Milk, R.T., acid 0.8	? d.	Marsh	1918
MILK PRODUCTS			
Sh. dysenteriae			
Buttermilk	15 d.	Stever	1941

TABLE F10—Continued

The Survival of Shigella Species in Food

Factor(s)	Survival	Reference	
MILK PRODUCTS (Continued)			
Butter	18 d.	Berry	1927
Curds	Do not survive	Bhat	1949
Fresh butter	Inoc. agar cult. ground in agar mortar	Pfuhl	1902
Gervais cheese	Inoc. agar cult., 9 d.	Pfuhl	1902
Sweet and sour milk curds, pH 4.2–4.7	Inoc. 4 drops of cult. Recov. 0, <4 hr.	Panja	1945
Sh. paradysenteriae			
Curds	Do not survive	Bhat	1949
Buttermilk	15 d.	Stever	1941
EGGS			
Sh. paradysenteriae			
Albumin balls, 17–20 C	1 d. or 1 mo.	Frost	1905
Frozen eggs, –9 C.	3 mo.	Hartsell	1951
MEAT			
Sh. dysenteriae			
Bacon and sausage, R.T.	>3 wk.	Buchanan	1918
CEREALS			
Sh. dysenteriae			
Bread after baking, R.T.	30 hr.	Alves	1935
Crust of rye bread	20 d. alive	Bachmann	1943
Crust of rye bread, R.T.	66 d. dead	Bachmann	1943
Bread, rice, 17–20 C.	1 d. or 1 mo.	Frost	1905
Bread, R.T.	11 d.	Stanley	1930
Sh. paradysenteriae (Flexner)			
Bread after baking, R.T.	30 hr.	Alves	1935
Crust of rye bread	23 d. alive	Bachmann	1943
Crust of rye bread, –5 to –25 C.	45 d.	Bachmann	1943
Sh. paradysenteriae (Sonne)			
Crust of rye bread	Over grown with spores	Bachmann	1943
VEGETABLES			
Sh. dysenteriae			
Soy bean sauce, 25–17 C.	Inoc. 1 loop, >24 hr.	Wang	1945
Soy bean sauce, 36–38 C.	Inoc. 1 loop, <24 hr.	Wang	1945
FRUITS			
Sh. dysenteriae			
Orange juice, –40 C.	170 hr.	Beard	1932
Sh. paradysenteriae (Sonne)			
Tomato surface	48 hr.	Johnston	1935
Tomato tissue	10 d.	Johnston	1935
Tomato	Inoc. artificial, 6 d.	Johnston	1935
Apple	Inoc. artificial, 8 d.	Johnston	1935

TABLE F11

The Survival of Streptococcus Species in Food

Factor(s)	Survival	Reference	
MILK			
Streptococcus cremoris			
Sterilized milk, 30 C.	2 d.	Mattick	1946
Streptococcus fecalis			
Milk, 62.8 and 37 C.	Young cells more resistant than mature	Stark	1929
Streptococcus spp.			
Milk, 110 C.	Recov. 4 hr.	Belin	1933
Milk, ice box	17 d.	Berry	1937
Sour milk, 30 C.	48 hr.	Davis	1914
Dried milk	The lower the humidity the longer the survival	Watts	1945
Cultures 55:			
Milk of resistant cow	Inoc. 6336, Recov. 9185, 8 hr.	McCullough	1945
Boiled milk of resistant cow	Inoc. 7260, Recov. > 20160, 8 hr.	McCullough	1945
Milk of young cow early in first lactation period	Inoc. 5356, Recov. 22464, 8 hr.	McCullough	1945
Boiled milk of same cow	Inoc. 5068, Recov. > 36992, 8 hr.	McCullough	1945
Freshly isolated strains:			
Milk of resistant cow	Inoc. 7168, Recov. 41320, 8 hr.	McCullough	1945
Boiled milk of resistant cow	Inoc. 7296, Recov. > 16588, 8 hr.	McCullough	1945
Raw milk of young cow early in first lactation period	Inoc. 1344, Recov. 19120, 8 hr.	McCullough	1945
Boiled milk of same cow	Inoc. 1197, Recov. > 30538, 8 hr.	McCullough	1945
DAIRY PRODUCTS			
Streptococcus pyogenes			
Butter	17 d.	Berry	1927
Limburger cheese, moisture content, 42.8	28–51 d.	Yale	1940
Limburger cheese, moisture content, 49.3	9–14 d.	Yale	1940
Cheddar cheese, cured at 45 F.	>18 wk.	Yale	1940
Cottage cheese, low temp.	Not recovered	Yale	1940
Streptococcus fecalis			
Cheddar cheese, 50 F.	Inoc. 300/ml., Recover. 11×10^6/ml., 180 d.	Kosikowsky	1948
Cheddar cheese, 60 F.	Inoc. 300/ml., Recov. 13×10^6/ml., 180 d.	Kosikowsky	1948
Streptococcus spp.			
Butter, from infected cow, salted	6 mo.	Bryan	1944
Butter, from infected cow, unsalted	6 mo.	Bryan	1944
Ice cream	18 d.	Davis	1914
Ice cream at 20 C.	No growth	Davis	1914
Ice cream at 26 C.	Growth in 20 hr.	Davis	1914

TABLE F11—Continued

The Survival of Streptococcus Species in Food

Factor(s)	Survival	Reference	
DAIRY PRODUCTS (Continued)			
Streptococcus spp.			
Ice cream refrigerator	Inoc. 45, Recov. 12 hr. 34,695 org.	Pennington	1907
Ice cream R.T.	Inoc. 70, Recov. 34,491, 12 hr.	Pennington	1907
Ice cream incubator	Inoc. 18, Recov. 85,844.4, 12 hr.	Pennington	1907
EGGS			
Streptococcus spp.			
Egg white, 50 C.	Recov. 6, 4 hr.	Belin	1933
MEAT			
Streptococcus pyogenes			
Sausage, 4 d. drying	13 d.	Mueller-Claus	1938
Lobster meat	May remain alive in salad a considerable time	Scamman	1927
Streptococcus viridans			
Potted meat	Inoc. 30–5000/gm. meat, Recov. $30x10^6$ org., 60 d.	Surgella	1945
VEGETABLES			
Streptococcus fecalis			
Veg., -20 C.	Recov. 89% out of 70 in 1 yr.	Burton	1949

TABLE F12

The Survival of Vibrio Species in Food

Factor(s)	Survival	Reference	
MILK			
Vibrio comma			
Milk, R.T.	11–63 hr.	Alessandrini	.....
Milk, 37 C.	6–8 hr.	Alessandrini	.....
Milk, boiled and uncovered	5–8 d.	Alessandrini	.....
Sour milk and cream	Lethal effect	Grattan	1939
Milk, 7–22 C.	6 d.	Heim	1889
Sterile milk, 22 C.	6–9 hr.	Lazarus	1890
Sterile milk, 35 C.	12–24 hr.	Lazarus	1890
Sterile milk, 62–70 C.	1 d.	Lazarus	1890
DAIRY PRODUCTS			
Vibrio comma			
Curds	Do not survive	Bhat	1949
Cheese	8–15 hr.	Genevray	1938
Butter, R.T.	21 d.	Grattan	1939
Butter, 37.5 C.	32 d.	Heim	1889
Curds, 35 C., weakly acid	0 d.	Heim	1889
Whey, 35 C., weakly acid	2 d.	Heim	1889
Cheese, 35 C.	1 d.	Heim	1889
Cheese	4–5 wk.	Hesse	1889

TABLE F12—Continued

The Survival of Vibrio Species in Food

Factor(s)	Survival	Reference	
DAIRY PRODUCTS (Continued)			
Butter (English), 15 C.	>38 d.	Pullinger	1938
Butter (English), 3 C.	>98 d.	Pullinger	1938
Sweet and sour curds, pH 4.2–4.7	Inoc. 4 drops cult., Recov. 0, 5 min.	Panja	1945
EGGS			
Vibrio comma			
Salted salmon eggs, 23–33 C.	Inoc. 0.1 cc., Recov. 0, 2 d.	Tetsumoto	1930
Grey mullet eggs, 23–33 C.	Inoc. 0.1 cc., Recov. 0, 3 d.	Tetsumoto	1930
Cod eggs, 23–33 C.	Inoc 0.1 cc., Recov. 0, >8 d.	Tetsumoto	1930
Salted sea-urchin eggs, 23–33 C.	Inoc. 0.1 cc., Recov. 0, 12 hr.	Tetsumoto	1930
MEAT			
Vibrio comma			
Fish cooked in acid	4 d.	Arguelles	1927
Fish boiled in salt water	6 d.	Arguelles	1927
Fish washings	<1 d.	Arguelles	1927
Sterile fish washings	6 d.	Arguelles	1927
Sterile fish extract	125 d.	Arguelles	1927
Salted fish cooked	1 d.	Arguelles	1927
Salted fish uncooked	<1 d.	Arguelles	1927
Uncooked small shrimp	6 d.	Arguelles	1927
Cooked small shrimp	4 d.	Arguelles	1927
Fresh cattle, blood sausage, ham broth	45 wk.	Hesse	1889
Meat	Well	Lal	1926
Fat	Poor	Lal	1926
Salmon skin 22.5–33 C.	18 hr.	Tetsumoto	1930
Salmon ventral, 22.5–33 C.	18 hr.	Tetsumoto	1930
Salmon flesh, 22.5–33 C.	18 hr.	Tetsumoto	1930
Salmon skin (ster.), 23–33 C.	26 hr.	Tetsumoto	1930
Salmon ventral flesh (ster.), 22.5–33 C.	19 hr.	Tetsumoto	1930
Salmon flesh (ster.), 22.5–33 C.	20 hr.	Tetsumoto	1930
Trout skin, 22.5–33 C.	26 hr.	Tetsumoto	1930
Trout ventral flesh, 22.5–33 C.	18 hr.	Tetsumoto	1930
Trout flesh, 22.5–33 C.	18 hr.	Tetsumoto	1930
Trout skin (ster.), 22.5–33 C.	25 hr.	Tetsumoto	1930
Trout flesh (ster.), 22.5–33 C.	18 hr.	Tetsumoto	1930
Aramahi salmon skin, 22.5–33 C.	38 hr.	Tetsumoto	1930
Aramahi salmon flesh, 22.5–33 C.	26 hr.	Tetsumoto	1930
Cod, 22.5–33 C.	8 hr.	Tetsumoto	1930
Ham, 22.5–33 C.	26 hr.	Tetsumoto	1930
Yellow fish skin, 22.4–33 C.	27 hr.	Tetsumoto	1930
Yellow fish flesh, 22.5–33 C.	27 hr.	Tetsumoto	1930
Mackerel skin, 22.5–33 C.	27 hr.	Tetsumoto	1930

TABLE F12—Continued

The Survival of Vibrio Species in Food

Factor(s)	Survival	Reference	
MEAT (Continued)			
Vibrio comma			
Mackerel flesh, 22.5–33 C.	25 hr.	Tetsumoto	1930
Mackerel pike, 22.5–33 C.	26 hr.	Tetsumoto	1930
Sardine, 22.5–33 C.	25 hr.	Tetsumoto	1930
Sea bream skin, 22.5–33 C.	32 hr.	Tetsumoto	1930
Sea bream flesh, 22.5–33 C.	32 hr.	Tetsumoto	1930
Horse mackerel, 22.5–33 C.	38 hr.	Tetsumoto	1930
Flying fish	38 hr.	Tetsumoto	1930
Bacon	40 hr.	Tetsumoto	1930
Tuna	2 d.	Tetsumoto	1930
Sword fish	3 d.	Tetsumoto	1930
Trout	2 d.	Tetsumoto	1930
Shrimp	3 d.	Tetsumoto	1930
Salted cod, 16–27.5 C.	Recov. 0, 12 hr.	Tetsumoto	1930
Salted salmon, 16–27.5 C.	Recov. 0, 36 hr.	Tetsumoto	1930
Aramahi salmon flesh, 16–27.5 C.	Recov. 0, 25–40 hr.	Tetsumoto	1930
Mackerel, 16–27.5 C.	Recov. 0, 25–40 hr.	Tetsumoto	1930
Sardine, 16–27.5 C.	Recov. 0, 25–40 hr.	Tetsumoto	1930
Flying fish	Recov. 0, 25–40 hr.	Tetsumoto	1930
Sardine (oiled), 19–34.5 C.	Inoc. 0.1 cc., Recov. 0, 33 d.	Tetsumoto	1930
Sardine (tomato), 19–34.5 C.	Inoc. 0.1 cc., Recov. 0, 31 d.	Tetsumoto	1930
Sardine (yamatoni), 19–34.5 C.	Inoc. 0.1 cc., Recov. 0, 20 d.	Tetsumoto	1930
Bointo (yamatoni), 19–35.5 C.	Inoc. 0.1 cc., Recov. 0, 6 d.	Tetsumoto	1930
Salmon, 19–34.5 C.	Inoc. 0.1 cc., Recov. 0, 27 d.	Tetsumoto	1930
Whitebait, 19–34.5 C.	Inoc. 0.1 cc., Recov. 0, 19 d.	Tetsumoto	1930
Crab, 19–34.5 C.	Inoc. 0.1 cc., Recov. 0, 18 d.	Tetsumoto	1930
Shrimp, 19–34.5 C.	Inoc. 0.1 cc., Recov. 0, 13 d.	Tetsumoto	1930
Sea-ear, 19–34.5 C.	Inoc. 0.1 cc., Recov. 0, 25 d.	Tetsumoto	1930
Scallop, 19–34.5 C.	Inoc. 0.1 cc., Recov. 0, 22 d.	Tetsumoto	1930
Beef yamatoni, 19–34.5 C.	Inoc. 0.1 cc., Recov. 0, 12 d.	Tetsumoto	1930
Cuttle fish, 22.5–27.5 C.	Inoc. 0.1 cc., Recov. 0, 5 hr.	Tetsumoto	1930
Fukujinzuke, 19–34.5 C.	Inoc. 0.1 cc., Recov. 0, 6 hr.	Tetsumoto	1930
Control in saline, 19–34.5 C.	Inoc. 0.1 cc., Recov. 0, 6 d.	Tetsumoto	1930
Fish, 5–13 C., natural oysters and clams, 22 C., ster. with steam	8 d.–2 wk.	Tohyama	1925
Oysters and clams, 0–5 C.	40 d.	Tohyama	1925

TABLE F12—Continued

The Survival of Vibrio Species in Food

Factor(s)	Survival	Reference	
MEAT (Continued)			
Vibrio comma			
Fish meat, 80 C.	2 min.	Tohyama	1925
Filsh meat, 70 C.	3 min.	Tohyama	1925
Fish meat, 60 C.	7.5 min.	Tohyama	1925
Fish meat, hottest day	7–10 d.	Tohyama	1925
Fish meat, 3–8 C.	2 wk.	Tohyama	1925
SAUCES			
Vibrio comma			
Nudu-mam sauce	6 hr.	Genevray	1938
Shrimp paste	5 hr.	Genevray	1938
Fermented soy sauce	<1 hr.	Genevray	1938
Soy bean sauce, 25–17 C.	Inoc. 1 loop, Recov. 0, >24 hr.	Wang	1945
Soy bean sauce, 36–38 C.	Inoc. 1 loop, Recov. 0, <24 hr.	Wang	1945
CEREAL			
Vibrio comma			
Soy bean milk	8–15 hr.	Genevray	1938
VEGETABLES			
Vibrio comma			
Potatoes	4–5 wk.	Hesse	1889
Veg.	Well	Lal	1926
FRUITS			
Vibrio comma			
Watermelon, pH 4.7	4 d.	Bharucha	1938
Watermelon	Inoc. artificial, 6 d.	Bharucha	1938
Grapes	4 d.	Dobrosklonsky	1911
Berries (inside)	24 hr.	Dobrosklonsky	1911
Grape stems	12 d.	Dobrosklonsky	1911
Grapes (inside)	24 hr.	Dobrosklonsky	1911
Fresh lime, pH 4.4	30 min.	Panja	1945
GENERAL			
Vibrio comma			
Human food	6 d.	Vasquet-Colet	1924
Vibrio sp.			
Skim milk, frozen, dried at 100 mm. press.	681 d.	Stockton	1950

TABLE F13

The Survival of Virus in Food

Factor(s)	Survival	Reference	
MILK			
Polio virus			
Milk	Polio virus can stand more heat in milk than water	Lawson	1947

TABLE F13—Continued

The Survival of Virus in Food

Factor(s)	Survival	Reference	
MILK (Continued)			
Foot and mouth disease virus			
Milk	Present	Jansen	1942
DAIRY PRODUCTS			
Polio virus			
Butter, ice box	91 d.	Kling	1931
EGGS			
Newcastle disease virus			
Eggs, 36 C., incubator	Up to 126 d.	Olesiuk	1951
Eggs, R.T., 20–30 C.	Up to 235 d.	Olesiuk	1951
Eggs, hen house, 36 C.	255 d.	Olesiuk	1951
Eggs, ice box, 3–6 C.	538 d.	Olesiuk	1951
Foul-pox virus			
Chicken eggs, in dry state	Active at 3598 d.	Beaudette	1948
Duck eggs, in dry state	Active at 1928 d.	Beaudette	1948
Pigeon-pox virus			
Chicken eggs, in dry state	Active at 3605 d.	Beaudette	1948
Duck eggs, in dry state	Active at 1099 d.	Beaudette	1948
Jap. B encephalitis virus			
Eggs, 4 C.	6 hr.	Morgan	1946
MEAT			
Foot and mouth disease virus			
Beef, –20 C., thawed in buff. phosphate soln. at 37 C.	4 mo.	Henderson	1948
Beef, –4 C.	24 hr.	Henderson	1948
CEREAL			
Foot and mouth disease virus			
Chopped hay, R.T.	15 wk.	Anon.	1927
Bran, R.T.	20 wk.	Anon.	1927
Hay and bran, dried	15–20 wk.	Burbury	1928
Hay (saliva on it)	>1 mo.	Krueger	1942
N. d. virus			
Mash, pH 5.5, 37 C.	56 d.	Olesiuk	1951
Mash, pH 5.5, 20–30 C.	94 d.	Olesiuk	1951
Mash, pH 5.5, 11–36 C.	172 d.	Olesiuk	1951
Mash, pH 5.5, 3–6 C., and –26 C.	>538 d.	Olesiuk	1951
FRUITS			
Polio virus			
Unwashed fruits and veg.	Present	Gebhart	1946

TABLE F14

The Survival of Yeast and Mold in Food

Factor(s)	Survival	Reference
MILK		
Yeast		
Milk, –21 to –78 C.	Inoc. 100,000/ml., more resistant to freezing than thawing	Lund

TABLE F14—Continued

The Survival of Yeast and Mold in Food

Factor(s)	Survival	Reference	
DAIRY PRODUCTS			
Mold			
Margarine	Viable 42%	Foltz	1951
Yeast			
Margarine	Viable 46%	Foltz	1951
VEGETABLES			
Mold			
Veg., frozen	90% killed	Magoon	1932
Veg., below freezing	16 mo.	Tanner	1931
Yeast			
Veg., frozen	90% killed	Magoon	1932
FRUITS			
Mold			
Fruit juices, –23.3 C.	3 yr.	Tanner	1934
Good oranges, 17.8 C.	Inoc. 10,900/cc., Recov. 2,600/cc., 7 mo.	Wolford	1948
Soft rotten oranges, 17.8 C.	Inoc. 26,500,000/cc., Recov. 990,000/cc., 8 mo.	Wolford	1948
Strawberries, –9.4 C., sealed tins	3 yr.	Smart	1934
Yeast			
Good oranges, 17.8 C.	Inoc. 10,900/cc., Recov. 2,600/cc., 7 mo.	Wolford	1948
Soft rotten oranges, 17.8 C.	Inoc. 26,500,000/cc., Recov. 990,000/cc., 8 mo.	Wolford	1948
Grape juice, freezing	18 wk.	Tanner	1944
Strawberries, –9.4 C., sealed tins	3 yr.	Smart	1934
BEVERAGES			
Yeast			
Beer, freezing	Cells remain active	Melsens	1870
Beer	15 yr.	Tanner	1944
GENERAL			
Yeast			
Food, –9.4 C.	3–15 mo.	Smart	1936
Syrup, very dry condition isolated from acid syrup	Several wk.	Owen	1948

SUMMARY OF ABBREVIATIONS USED IN TABLES

alk.	alkaline	org.	organism
avg.	average	path.	pathogenic
C.	Degrees centrigrade	physiol.	physiological
Col.	Colonies	ppm.	parts per million
conc.	concentration	ppt.	precipitate
cont'd, cont.	continued	R.H.	Relative humidity
ct.	count	R.T.	Room temperature
cult.	culture	recov.	Recovered
d., ds., das.	day or days	refrig.	refrigeration
Dessic.	Desiccate	sec.	second
dil.	dilution	sensit.	sensitization

F.	Degrees fahrenheit	soln., sol'n.	solution
fl.	fluid	spp.	species
G.P.	Guinea pig	str.	strain
gel.	Gelatin	susp., susp'n.	suspension
h., hrs.	hour or hours	T.B., tb	tuberculosis
inc.	increase	temp.	temperature
Inoc., Innoc.	Inoculate	U.V., U.v., UV	Ultra violet
irrad.	irradiated	wks.	weeks
Lg.	Large	X	times
max.	maximum	yr., yrs.	year or years
med.	medium	>	greater than
met.	methyl	<	less than
min.	minute or minutes	+	present; plus
mos.	months	0	none
mult.	multiplied	—	minus

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ON THE PHILOSOPHY OF RESEARCH

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To set the stage for discussion, I should like to present a statement of E. A. Burtl's which appeared in his work, The Metaphysical Foundations of Modern Science, "The world-view of any age can be discovered in various ways, but one of the best is to note the recurrent problems of its philosophers. Philosophers never succeed in getting quite outside the ideas of their time so as to look at them objectively—this would, indeed, be too much to expect. Neither do maidens who bob their hair and make more obvious their nether bifurcation see themselves through the eyes of an elderly Puritan matron. But philosophers do succeed in glimpsing some of the problems involved in the metaphysical notions of their day and take harmless pleasure in speculating at them in more or less futile fashion." But lest you become alarmed. I hasten to add that my emphasis will not be on metaphysics. My approach to some problems of research will be a much more humble one—and at the same time more *immediately* practical.

The discussion includes many quotations. This deserves explanation—The views of every person capable of expressing views are based upon experience. Some of this experience is personal; much of it is secondhand. In the latter case, depending upon the individuality of the viewer, selection may be very critical and thus very restricted. In any event it is certain that one's views on such a broad subject as research will represent a summation of the views of many persons. Some of these views will have been accepted directly, others will have been rejected (possibly accepted *in reverse*) or modified in accordance with one's own experience and philosophy.

Many seem to consider it a sign of personal inadequacy to quote directly the views of others even though their own thoughts were derived from these views. There is a fault in this. The recipient is denied the opportunity of a personal and unique re-

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action which might have been quite different from that of the speaker. Obviously the *selection* of excerpts in itself is an expression of individuality and hence is a form of editorializing. But to utilize the critical views of others without presenting them in unaltered form is a very much worse form of editorializing.

"The wise men of antiquity, when they wished to make the whole world peaceful and happy, first put their own states into proper order. . . . Before putting their states in proper order, they regulated their own families. . . . Before regulating their families, they regulated themselves. . . . Before regulating themselves, they tried to be sincere in their thoughts. . . . Before being sincere in their thoughts, they tried to see things exactly as they really were."—Confucius.

Seeing things as they really are is that difficult task which may be wholly, or at least in part, accomplished by research.

What is research? I think that it is much more than a careful search or even a searching again. Henry H. Donaldson says, "Research is a frame of mind. A man . . . if he examines his world in a questioning spirit, if he carries with him not only conclusions, but the observations on which they rest, if he refuses to pound square facts into round holes that he happens to have in hand, he has attained illumination. The spirit of research is nothing mysterious or remote, it is everyday hard sense." Iago Galdston says, "Research is distinguished by the quality and nature of its problems. Research does not merely aim to add to the sum of our knowledge, but to provide us with understanding and insight into dynamic interrelationships." Actually, research is a *form of inquiry*—a form of inquiry that is especially applicable to science. Scientific research is more than ever important today because, in the words of Bertrand Russell, "Science used to be valued as a means of getting to know the world; now, owing to the triumphs of technique, it is conceived as showing how to *change* the world." It is becoming increasingly evident that if we are not alert, this change may be very much for the worse.

Seeing things as they really are is perhaps the most important function of research. This requires a frame of mind which is not possessed by everyone. Bacon said, "The mind is not like a plane mirror which reflects the images of things exactly as they are; it is like the mirror of an uneven surface, which combines its own figure with the figures of the objects it represents."

Knowledge is a function of the knower, necessarily modified by his philosophy, by his experiences and by his immediate objectives.

The essence of the scientific method is inductive reasoning, *i.e.*, generalizing from the particular. Most scientists have an "unconscious love of uniformity" which may lead them "to find a greater degree of order, simplicity and regularity than is actually indicated by observation" (Westaway) and thus to be diverted from the truth. This is one of the great pitfalls which the researcher must avoid. Hadfield and Garrod put this very well in their "Recent Advances in Pathology"—"No service to biology can be greater than the exposure of fundamental laws, but it is equally a disservice to unify where no unity exists and to try to explain on a single basis phenomena of different nature and cause." Bertrand Russell was thinking along similar lines when he said, "So long as men are not trained to withhold judgment in the absence of evidence, they will be led astray by cocksure prophets, and it is likely that their leaders will be either ignorant fanatics or dishonest charlatans. To endure uncertainty is difficult, but so are most of the other virtues."

How does one keep from going astray in this direction? It *is* difficult and requires ceaseless vigil. The researcher must not be misled by his own or anyone else's interpretations of data. He must not confuse the hypothesis with the fact. Wilson summarized the problem very well when he said, "Although the scientist uses his mind to imagine ways in which the world might be constructed, he knows that only by looking at reality can he find out whether any of these ways correspond with reality. He rejects *authority* (his own as well as that of others—H.C.H.) as an ultimate basis for truth." To put it another way, *What is NATURE'S method?*

An enormous amount of emotional and physical energy has been expended in defense of false doctrines and hypotheses by those so misguided as to believe that their own ideas shape the truth—by those who have failed to realize that "a clash of doctrines is not a disaster—it is an opportunity" (Whitehead). Today, happily, the penalty for questioning *scientific* doctrine is not great (though even today a scientist may lose his means of livelihood and have difficulty in obtaining suitable recommendations for another position). But it was not always thus—in 1591

members of the University of Pisa and lay onlookers assembled at the base of the leaning tower one morning to witness an experiment which was to be conducted by a very radical young professor whose name was Galileo. Galileo proposed to *test* rather than to philosophize further on the time honored dogma which stated that bodies fall through air at a speed directly proportional to their weight. The heavy and the light ball which Galileo dropped simultaneously from the Tower fell together and struck the earth together for all to see. The obvious (to us) solution of this problem was not a solution at all to many of the "learned" men who had come to witness the exhibition. Among this group the consensus of opinion was, "This meddlesome man must be suppressed—such disregard of authority is dangerous."

Freedom and authority in research could well be the title of a very large monograph. This is in part because there is no such thing, practically speaking, as absolute freedom. Although not directly pertinent to our subject, Whitehead's comment on the aims of education does have a bearing here—"The only avenue towards wisdom is by freedom in the presence of knowledge. But the only avenue towards knowledge is by discipline in the acquiring of ordered fact. Freedom and discipline are the two essentials of education." Paraphrasing this remark, I believe that freedom and authority are two essentials of effective research. What is the nature and the limit of the authority? Freedman has effectively answered this difficult question in a single sentence—". . . authority must be a modest guide, and not a judge from whom there is no appeal." What is the nature and the limit of the freedom? This question too can be simply answered. A researcher should be free to seek the truth and to express his views about it. He should be free to combat all dogma and authority whether of the left or of the right.

The symbolism of science, which gives deep insight into the meaning of science and its values, has been simply expressed by Wendell Johnson in his "People in Quandaries." This symbol, in form of a diagram familiar to us all, ". . . represents what any scientist strives to express: a variation of one kind, a variation of another kind, and the relationship between them." This does very well to help us understand the meaning of science, but is there a symbol which can help us to understand or guide us

in applying the research method? I have devised such a symbol-diagram which I call the Wheel of Research. It begins with the observation that two factors seem to have dependence upon each

other—that the value of one is apparently influenced by the value of the other. The second step (or quarter turn of the wheel) is to make certain that this observation is reliable, that it did not occur merely by chance—to determine with certainty that a relationship *does* exist (and some of the conditions which influence it). From these data, as the wheel makes another quarter turn, there is opportunity for *synthesis*, that is, the conception of a new principle. This is the ultimate goal of the researcher. This depends upon correlation of many observations—correlation of data which must have been gathered (and recorded) with precision and clarity. Almost all established principles of science have begun as hypotheses, as expressions of ideas that *might* be true. And so we are led to another quarter turn of the wheel and the testing, with proof (or disproof) of our hypothesis—its conversion into principle—the synthesis completed. But in the course of our testing, our inquiry as to whether or not $X + Y = Z$, we may observe (if we are alert) a *new* relationship between two *new* variables, that A is a function of B, and the Wheel of Research begins its cycle anew.

Today, as state governments and private industries are realizing the great value of the research method and providing increasing financial stimulus for research, there are more opportunities than ever before and consequently more research is be-

ing done than ever before. But many, many answers to scientific questions, though accurate, clearly stated, and readily available in the scientific literature, are relatively useless because *no one is interested in the particular questions involved.* Some of these answers may some time find important application in areas that we cannot now visualize. Unfortunately most of them won't. Thus, not only the right answer but the *right question* is important. I cannot give any formula which enables one to ask the right question. Perhaps it is this, more than any other element of research, that requires at least a spark of genius. The *manner* in which the question is asked is very important too. Einstein put it, "The formulation of a problem is often more essential than its solution, which may be merely a matter of mathematical or experimental skill." The *method* of the inquiry, fortunately is somewhat easier.

It has been said that there are two ways of detecting something that no one can see: one is to aim at the finest detail, to get as close to the object as possible, to use the best analytical instruments; the other is merely to look at it from a new angle, where it shows hitherto unexposed facets. If we accept the view of Laplace, that "to discover is to bring together two ideas which were separate" there is a third way of detecting something that no one *has seen*—by applying an unbiased mind and a fresh point of view to the contemplation of data already gathered. I think it would not be far wrong to say that an unbiased mind and a fresh point of view are the most important "tools" of research. Almroth Wright spoke in a similar vein when he said, ". . . trustworthy conclusions are reached only when we have been compelled to look at the facts from opposite points of view."

In methodology, as in most things, a happy medium is to be sought, and this requires compromise. In the methodology of research, the compromise is between (a), the time and the effort required to perform experiments directed towards securing necessary information and (b), the degree of accuracy with which the experiments must be done to insure that the information will be valid. It is important to employ method and technique which will give data sufficiently accurate to fall within the limits of allowable error, *but not more.* The value of simple techniques of "rough utility" which compensate for their lack of great accuracy by ease of rapid application is dramatically evident from

the work of Almroth Wright. The simple methods which Wright developed were considered by him to be so important that he devoted an entire book to their description—"The Technique of the Teat and the Capillary Glass Tube." It is true that there are many problems which require elaborate apparatus, attention to minute detail, and maximum accuracy, but most problems don't, at least not in their formative stage, and it is a serious mistake to waste time, energy, material, and equipment in securing a greater degree of accuracy than is needed. With proper judgment, one may do less to achieve more.

Another error which commonly causes great inefficiency, or even failure, is distraction from the primary purpose or objective. Dr. Paul Cannon, my old chief at the University of Chicago, would frequently admonish us to keep our eye on the target. This advice is as good today as it was then. In my own laboratory I have mounted on the wall as a constant reminder the words of Ralph M. Evans—"*Data, however precise, have no meaning unless they are based directly on the phenomena under observation.*"

To the extent that researchers differ in personality, the techniques which they can use to best purpose will also differ. Thus each must learn what he can from another, but must not try to force his pattern of behavior into the mould of another. Basic differences in point of view among eminent researchers are evident from many accounts; the following two illustrations will suffice here: "During my work as a student I repeatedly tried to follow up certain lines of thought by experiment. But I did not succeed in carrying out a single one of my ideas in this way. Soon, however, I came to try the other way round, that is to say, not to give directions to Nature but simply try to analyze striking experimental facts which were difficult to understand and by so analysing to find the laws which governed the action." Contrast this view of Paul Ehrlich's with that of Ramon y Cajal who said, "Hypothesis is our most valuable intellectual tool—a tool like all others, capable of becoming nicked and rusty, in need of continual repairs and replacement, but without which it would be almost impossible to carve out any kind of impression in the tough block of reality." Pasteur's statement represents a nice compromise between these two apparently divergent views—"Preconceived ideas are like searchlights which illu-

mine the path of the investigator and serve him as a guide to interrogate nature. They become a danger only if he transforms them into fixed ideas—this is why I should like to see these profound words inscribed on the threshold of all temples of science: 'The greatest derangement of the mind is to believe in something because one wishes it to be so.' " "The great art consists in devising decisive experiments, leaving no place to the imagination of the observer. Imagination is needed to give wings to thought at the beginning of experimental investigations on any given subject. When, however, the time has come to conclude, and to interpret the facts derived from observations, imagination must submit to the factual results of the experiments."

A final point which, in my opinion should be emphasized again and again is that the most successful researchers have *not* been required to sacrifice everything on the altars of science. They have been successful human beings too. Many of them have been deeply concerned with civic affairs and have had an important influence for good in social. economic and political matters. Nearly all the really accomplished scientists have inspired their colleagues and stimulated their students so that their scientific influence has extended well beyond the limits of their own research.

What of the future of biologic research? What lies ahead? What are our great opportunities? One of the very favorable aspects of our scientific age is that no one, not even the uninformed, believes that we've gone about as far as we can go. In our present state of scientific advancement we've reached just about high enough to see that the horizon is unlimited.

I'm not a prophet or seer—and my concluding remarks are probably similar to the ones that many of you would make were you in my position. Nevertheless, with your indulgence I shall tell you what I foresee of the future.

Of all the things of science, I am most impressed by the marvel of that toti-potential cell, the ovum! What mysterious forces control its growth?—its reproduction again and again and again? What is it that causes this reproduction to stop at the precise moment when the organism is complete? Why does not this growth continue to make giants of us all—microbes, mammoths —mice and men alike?

But we've considered only numbers and mass. DIFFERENTIATION is such a wondrous accomplishment as to be virtually beyond belief, let alone comprehension. The nature of those forces bound within the membrane of a single toti-potential cell, so small as to be invisible to the unaided eye, that leads to specialized development of parts and their organization to make so complex a structure as man, is perhaps the greatest mystery of all science. To think that within a single cell, quite undifferentiated and quite simple (to our uneducated eye) there is contained the pattern, and the means to build along this pattern not merely eyes, but blue or brown eyes—not merely hair, but hair that is red or black or yellow—straight or curly or kinky—hair that will begin to "fall out" at a relatively early age—or hair that will retain its luster late in life or hair that will become "prematurely" dry and grey. These things are determinable at this one cell stage as surely as is the four chambered character of our heart—the number and distribution of acid secreting cells in the stomach—the distribution of nerve cells and their interconnections so that precise sensory and motor functions can be carried out—and so that we can think—so so that we can have aspirations and hopes—and so that we may have a conscience as well as consciousness. And finally, so that we may produce cells which have all the potentialities of the cell from which we ourselves came.

Long ago we began to look critically at the individual and to evaluate his appearance and function—and this was the beginning of medicine. Then we began to examine critically the big parts, the organs—then the smaller parts, the tissues and the functional units of the organs, the liver lobule, the nephron, etc. After thousands of years, what an enormous achievement it was when Virchow focused attention on the *cell* as the basic unit—not just in health, but in disease too.

Today we are looking at cytoplasmic constituents and at parts of nuclei. We're vitally interested in mitochondria and chromosomes—and we are beginning to understand that we have barely scratched the surface. We have only just recently come to realize that cells are analagous to molecules. That just as in and *within* the atoms—there lies a whole new world of structure and force of infinite importance, so it is with the components of the cell.

If I may make another analogy, consider the astronomers—

their early observation that there were the "fixed" stars and then a few which moved independently—then Galileo and Copernicus and Kepler and Newton and some understanding and orderliness of knowledge. Then the great telescopes using first light, then radio waves—and the spectroscopes—and beginning knowledge of the immensity of things—and some appreciation of stellar evolution and understanding that our universe is a pin point in an enormous galaxy and that this galaxy is but one of many. Now consider what will happen to the science of astronomy when the first rocket ship lands on the moon and on Mars and on Venus. There will open new vistas of such broad proportions as to be virtually inconceivable now.

I believe that comparably broad vistas lie before us biologists and that they are contained within the cell—even within the parts we now toy with as an infant toys with his rattle.

ADSORPTION OF SULFUR-35 LABELED L-METHIONINE BY SERUM PROTEINS *IN VITRO*

WILLIAM C. LEVIN, JOHN E. PERRY, AND
T. G. BLOCKER, JR.*

Other investigators have used S-35 labeled L-methionine to study protein metabolism (Tarver *et al.*, 1942, 1948; Friedberg *et al.*, Harper *et al.*, 1947; Kelly *et al.*, 1950; Kinsell *et al.*, 1948, 1950; Knipping *et al.*, 1952; Mass *et al.*, 1948). After injection of the labeled methionine into the blood stream of living animals it has been observed that the radioactivity can be demonstrated in the plasma proteins (Blocker *et al.*, 1955; Harper *et al.*, 1947; Kinsell *et al.*, 1948, 1950; Levin *et al.*, 1955). It is generally believed that the radioactivity of the protein represents incorporation of the labeled methionine by newly synthesized protein molecules. This is supported by the fact that N-15 labeled glycine appears in the serum protein fraction in much the same manner as does labeled methionine (Schoenheimer *et al.*, 1942). The validity of this method may be questioned, however, on the basis that the radioactivity of the serum proteins may represent nonspecific adsorption of the labeled methionine by the protein molecule. This experiment was designed to determine whether adsorption does account for a significant amount of the radioactivity found in the serum protein in order to test the biological validity of the use of S^{35} labeled L-methionine as a means of following protein metabolism *in vivo*.

Materials and Methods. A measured amount of S-35 labeled L-methionine was added to 20 ml. of fresh human serum in a test tube and placed in a water bath at 37° C. In one series a "physiological" dose (1.3 μc., 37 μg.) of labeled methionine was added to the serum; *i.e.*, a dose which would approximate the concentration of labeled methionine in the blood immediately after injection in a human assuming that it became mixed and that no diffusion or utilization occurred. In another series a massive dose (130 μc., 3.7 mg.) was added; *i.e.*, 100 times the "physiological" dose. Aliquots were removed 15 minutes, 30 minutes, and every hour up to eight hours after the labeled

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methionine was added. The serum protein was precipitated with 20 per cent trichloroacetic acid, washed, dissolved, and reprecipitated three times. The reprecipitation was necessary to avoid errors due to occlusion of radioactive supernatant in the protein precipitate. The samples were filtered, weighed, and counted using a windowless gas flow counter. After corrections for radioactive decay and self-absorption the results were expressed as the per cent of the added dose adsorbed by the protein. These results were then plotted against time.

Results. The adsorption of the "physiological" dose is shown in Fig. 1 and the adsorption of the "massive dose" in Fig. 2. It

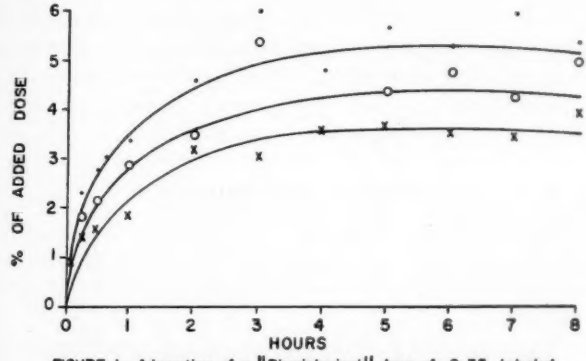

FIGURE 1- Adsorption of a "Physiological" dose of S-35 labeled L-Methionine by serum proteins in Vitro.

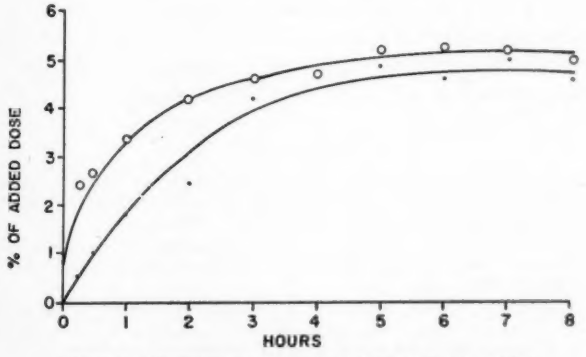

FIGURE 2- Adsorption of a "Massive" dose of S-35 labeled L-Methionine by serum proteins in Vitro.

is seen that in both instances the fraction of labeled methionine adsorbed never rose above 5 per cent of the added dose during the first 8 hours. The amount adsorbed at 15 minutes never exceeded a maximum of 2.5 per cent of the added dose.

From the results obtained it is demonstrated that no significant increase in adsorption is noted between 3 and 8 hours after mixing. The most critical part of the curve is during the first 5 hours following addition of the methionine, since it is during this time that radioactivity appears in the serum protein *in vivo* (Blocker *et al.*, 1954, 1955; Levin *et al.*, 1955). After 5 hours there is usually no increase in radioactivity of the protein. Since only about 5 to 19 per cent of the injected methionine is present in the total circulating blood 15 minutes after injection, the serum proteins are exposed to the free amino acid for only a brief interval of time. Therefore, the amount of *in vivo* adsorption that occurs during that time is probably less than the observed *in vitro* maximum of 2.5 per cent.

Summary and Conclusions

1. The maximum adsorption of S-35 labeled L-methionine by serum proteins at 37° C. *in vitro* is 2.5 per cent of total radioactivity of serum 15 minutes after mixing and 5 per cent 8 hours thereafter.

2. It is suggested that the amount of non-specific adsorption of L-methionine by serum proteins *in vivo* is no greater than that which occurs *in vivo* and is probably less.

3. It is probable that the appearance of radioactivity in serum proteins following administration of S-35 labeled L-methionine represents biosynthetic incorporation almost entirely and non-specific adsorption only to an insignificant degree.

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PULMONARY HYALINE MEMBRANE DISEASE OF INFANCY: A HISTOCHEMICAL STUDY

P. O'B. MONTGOMERY*

The exact nature and origin of the material which makes up the hyaline membrane in hyaline membrane disease of infancy is not known. Previous histochemical studies have identified some of the chemical constituents of the membrane. This paper presents the results of additional histochemical studies applied to the membranes of hyaline membrane disease.

Materials and Methods

Formalin fixed blocks of lung were selected from 6 cases of premature infants dying with hyaline membrane disease. The morphologic and clinical findings in these cases were identical to those described in the literature by Potter (1952). All of the histochemical procedures reported were performed on frozen sections of formalin fixed tissue. The procedures employed were performed as outlined by Pearse (1953) or as given by specific reference.

Results

The membranes were found to contain various lipid components as shown by positive staining with Oil red O, Sudan black B, Nile blue sulfate. These results indicate the presence of triglycerides, fatty acids, phospholipids, acetal lipids, phosphatides, triolein and oleic acids. The Nile blue sulfate stain was blue indicating the predominant presence of acidic lipids instead of triglycerides. It should be pointed out that the membranes were not homogeneous in their lipid content. Some portions of a single membrane gave a negative stain while other areas were moderately positive and in other cases, an entire membrane was negative.

All of the membranes contained free potassium as demonstrated by a positive reaction using MacCallum's method as

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modified by Gomori (1952), and protein bound sulfhydryl groups as shown by a positive reaction using the method of Barnett and Seligman (1952). In this latter procedure the membranes were red in color indicating the presence of a moderate number of protein bound SH groups. Free carbonyl groups were present as demonstrated by a purple color with the method of Ashbel and Seligman (1949). These groups were present in moderate amounts and the intensity of the color varied somewhat from membrane to membrane and to a lesser degree within any one membrane. The Periodic Acid Schiff procedure gave a positive reaction in the membrane when performed on frozen sections. When this procedure was performed on the identical tissue prepared by the conventional paraffin section technic, the results were negative, or occasionally equivocally positive. A positive reaction here is indicative of free aldehyde groups generally of mucopolysaccharide, polysaccharide, mucoprotein, glycoprotein, glycolipid and unsaturated lipid origin. In this instance the majority of these groups were incorporated in substances which were removed in the process of the paraffin section technic.

In localized areas the membrane contained additional substances. Fuelgen positive material (presumably DNA) occurred as small isolated spots scattered at random in the membrane or apparently attached to or incorporated in its surface. These spots are taken to indicate nuclear debris, the cellular origin of which was not determined. The membrane gave intermittently positive reactions for calcium using the Alizarin red technic. No single membrane was uniformly positive for calcium but all of the membranes contained positive areas. Best's mucicarmine stain gave positive results in selected areas indicating the presence of mucin. This material was most prominently located on the surface of the membrane next to the alveolar lumen, but it was likewise present in focal areas within the membrane itself. The membrane itself, was negative for glycogen by Best's technic, but loose fragments of material around the membrane and in the alveolar space were positive. Two technics were used to demonstrate tyrosine. With one, the Millon reaction, an equivocally positive result was obtained in the membrane itself. With the other, the xanthoproteic reaction, the result was entirely negative.

The membranes gave a negative reaction for or failed to take a stain with Congo red, Hgb, Fe, Vit. C, bile, indole, or non-specific esterase by the method of Nachlas and Seligman (1949) and for mucoproteins with the Bismark brown stain and for cholesterol and its esters using the Schultz reaction.

By the paraffin section technic the membrane stained red with hematoxylin and eosin, red with a slight blue cast with Mallory, light pink with Masson, and a granular black-brown with a thin pink alveolar surface with Verhoff's elastic tissue stain.

Discussion

Some of the ingredients and tinctorial properties noted in the hyaline membranes in this report have been previously reported. These have recently been summarized in a review by Tran-Dinh-De and Anderson (1953). The results obtained here are essentially in agreement with others with a few exceptions. Dick and Pund (1949) reported negative stains for mucus, a result with which we are not in agreement. The majority of other workers have limited their studies to the demonstration of lipid material as a constituent of the hyaline membrane.

More recently Gilmer and Hand (1955) have studied in some detail the nature of the Schiff positive materials identifiable in the membrane. They conclude that this material is composed of carbohydrate with a 1,2 glycol linkage. They consider this as proof that the membranes were not derived from vernix. They also reported a positive Millon reaction in the membranes, an observation which was not confirmed by this study.

The observations made in this study indicate that the hyaline membrane of premature infants contain a wide variety of substances. Because of the erratic nature of the distribution of many of the other materials it seems unlikely that they have their origin from such a relatively homogeneous substance as plasma. It appears more likely that the membranes represent aspirated material combined with autochthonous material of pulmonary origin, such as bronchiolar epithelium.

Summary

Hyaline membranes of premature infants have been subjected to a variety of histochemical procedures. The membranes have

been demonstrated to contain lipids, carbohydrates, free carbonyl groups, aldehyde groups, DNA, mucus, calcium, potassium and protein bound sulfhydryl groups. In view of the erratic distribution of many of the components it seems likely that the membranes are made up of aspirated material as well as some autochthonous debris.

I wish to thank Miss Nancy Arnold for the technical performance of the histochemical procedures.

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LARGE SCALE PAPER ELECTROPHORESIS

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During the course of a survey of the incidence of electrophoretically distinct hemoglobin abnormalities (1), the need arose for a paper electrophoresis apparatus capable of accommodating large numbers of samples simultaneously. The present report deals with a description of such an apparatus and of methods to facilitate large scale paper electrophoresis. These methods, permitting the preparation and analysis of about 50 hemoglobin samples in a day, or the analysis of serum samples, either alone or simultaneously with hemoglobin, are applicable to general clinical as well as to survey purposes.

Methods

Paper Electrophoresis Apparatus. The apparatus, shown in Fig. 1, is a modification of one previously described (2). Made of plexiglass, it contains two buffer chambers separated by a frame which holds a vertical center bar and a Y-shaped arm at each end. Grooves in the vertical bars support the center of 25 cm wide filter papers, and each arm of the Y contains three openings for insertion of rods over which three papers can be placed separately. The buffer chambers, with a total capacity of 3 l, are divided in halves lengthwise by a baffle to form paper and electrode compartments; each of the latter has its own cover, along the length of which is suspended a coiled platinum wire electrode. Another lid covers both paper chambers. Sheets of filter paper 25 cm wide and about 32 cm long are marked with lines to indicate the position of each sample. When three papers are used, the first is supported in the lowest grooves of the center bar, with the ends hanging over the lowest and innermost rods and held by surface tension against the medial walls of the buffer chambers. The paper becomes moistened by capillary action or, more rapidly, by application of buffer along the rods. When the paper is uniformly saturated with buffer, the hemo-

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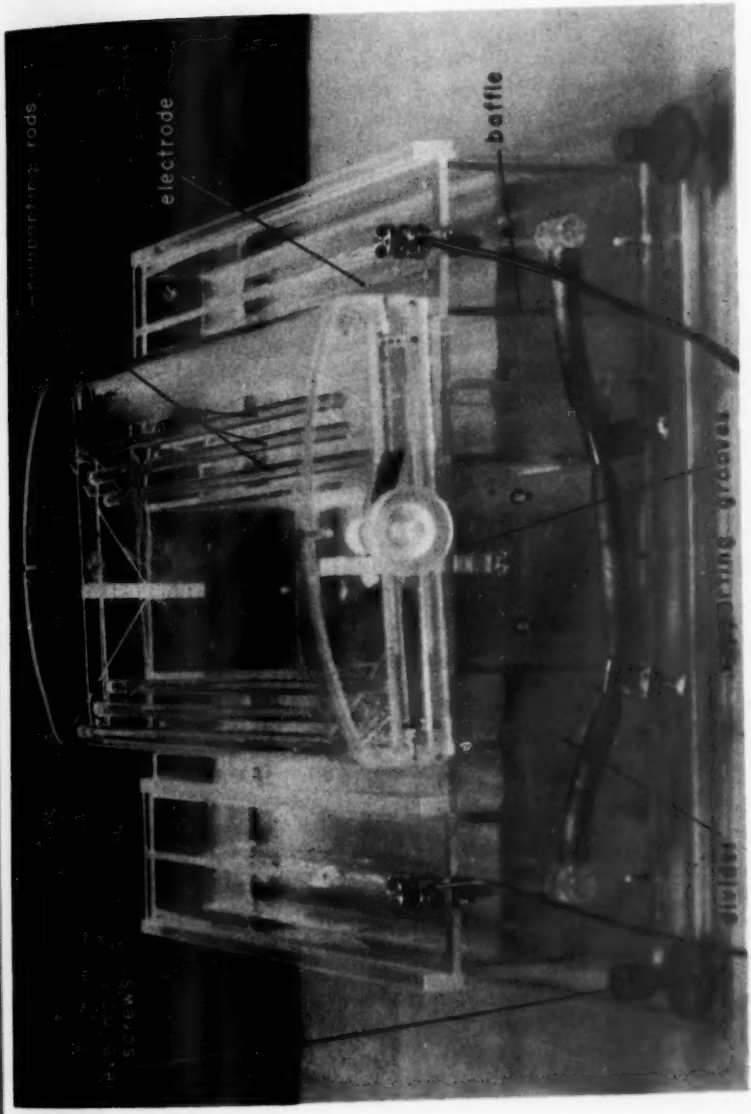

FIG. 1. Paper Electrophoresis Apparatus.

globin or serum aliquots are placed on the appropriate marks in the center. The second filter paper is then inserted into grooves about 2 cm above the first one and hangs over the middle bar, followed by the third paper, supported in grooves 2 cm higher, and hanging over the top and outermost rods. In each case, the paper is allowed to become moistened before the samples are applied. A divider in the center of the paper chambers facilitates separation of the second and third papers. If simultaneous serum analyses are desired, the serum samples are placed on the top paper. The papers are then covered with the lid and current is applied. The current may also be applied in the interval necessary for the upper papers to become wet. Under the stated conditions, the serum analyses are completed in about four hours. The current is then stopped and the paper with the serum samples is removed and dried in an oven at about 80° C. The cover is replaced and current is reapplied to the two papers containing hemoglobin samples for an additional period of about four hours. Of course, only one or two papers may be used, or serum analyses may be performed on all three papers, and all removed after four hours. Removal of the papers is accomplished by cutting the ends just below the supporting rods.

When the papers are placed in appropriately spaced grooves, the length of each paper between the points of immersion into buffer becomes approximately equal, so that each is subjected to similar potential gradients. In the present study, this distance was about 24 cm. Higher potential gradients can be obtained by supporting the paper in grooves closer to its supporting rods, thus shortening the distance along the paper from one buffer chamber to the other; or by selection of grooves farther from the rods this distance can be lengthened to provide a longer migration path. Veronal buffer pH 8.6 μ 0.05 is used; at the end of each run the electrodes are reversed, so that the same buffer may be re-used several times without significant changes.

Current is provided by a regulated power supply capable of delivering about 450v and furnished with a resistor of 10,000 ohms. With maximum voltage and three papers, there is an output of about 20–25 ma.* If hemoglobin samples are analyzed on all three papers, 400–450v are applied for 18–24 hours. With only one paper, this voltage is applied for about 6–8 hours, or,

* All measurements are taken before the current passes through the resistor.

for optimum separations, 200–300v for 24–30 hours. The routine analyses are made at room temperatures (occasionally as high as 35° C.), but for best results the apparatus is placed in a refrigerator and the door closed on the wires leading to the power source.

Preparation of Hemoglobin Samples. Hemoglobin samples are prepared by addition of one volume of water and one-fourth volume toluene to packed cells. After thorough mixing and standing for several hours or overnight, the tubes are centrifuged at 2,000 r.p.m. for about 15 minutes, the hemoglobin layer is drawn off, and if necessary, centrifuged again as before. Hemoglobin solutions of about 15 per cent are obtained by these procedures. At first the erythrocytes were washed three times with 0.85 per cent NaCl before hemolysis. However, this washing was time consuming, and was repeatedly found to produce no appreciable improvement in the results. Routine washing of erythrocytes was therefore omitted. Moderate variations in concentration were also found to have no appreciable effect on the results, so that the hemoglobin concentrations were ordinarily not determined. As a source of hemoglobin samples for purposes of the incidence survey, blood clots from the Serology Laboratory were found satisfactory. These were broken up with the aid of a glass homogenizer, treated with water and toluene and centrifuged as mentioned.

Application of Samples. Each of the 25 cm wide papers can accommodate 14 hemoglobin samples in 0.002 ml aliquots, or 18 samples in 0.001 ml aliquots. Usually, these aliquots are applied directly from a graduated 0.1 ml pipette; if semi-quantitative results are desired, uniform streaking of the sample is preferable. This streaking is accomplished by placing the sample on two 0.029 inch parallel stainless steel wires. An aliquot of 0.01 ml serum or 0.006 ml of about 15 per cent hemoglobin is applied to the wires (made fat free by dipping into acetone) from a fine tipped pipette, and is held on the wires by surface tension, until released by gentle pressure against the appropriate mark on the paper.

Evaluation of Samples. For hemoglobin analyses at least one control hemoglobin sample, usually from a subject with sickle cell trait, is applied to each paper, and the unknown specimens are evaluated in relation to this control. The relative positions of the better known types of hemoglobin, with the exception of

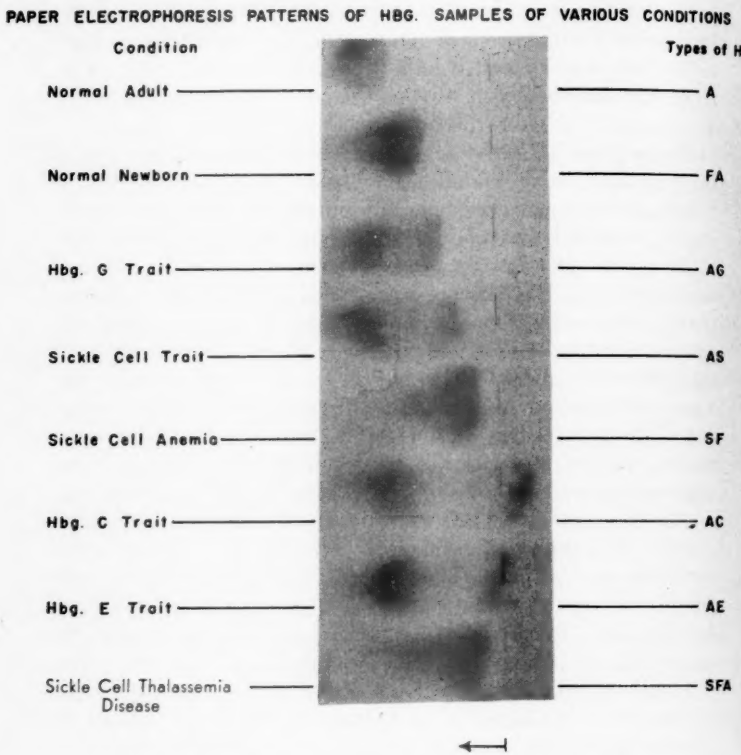

FIG. 2. One paper, at 250v for 30 hours, refrigerated.

the rare D (3) are shown in Fig. 2; in the order of the greatest distance of migration toward the anode these are: A, F, G, S, E and C.* The characteristic patterns of various conditions associated with these types are shown in Figs. 2 and 3.

In Fig. 3 are shown the three papers of an analysis in which aliquots of one serum sample were placed on the top paper and those of various hemoglobin samples in identical positions on the other two. In this analysis, 420v were applied, and the top paper with the serum samples was removed at the end of four hours;

* The hemoglobin E trait (4) sample was obtained from a Siamese individual currently residing here. The hemoglobin G trait sample was from the subject, J.R.A. in whom hemoglobin G was originally described. (5).

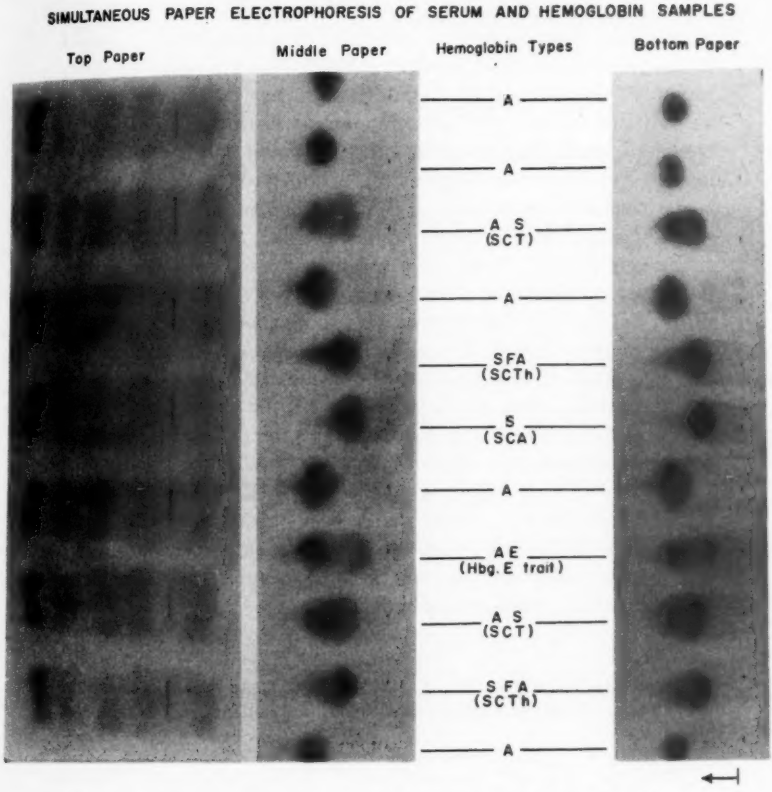

FIG. 3. Top paper contains aliquots of serum from a patient with third degree burns. Other two papers contain various hemoglobin samples applied in identical positions. Total duration of analysis, 7½ hours. Top paper removed at 4 hours. (See text.)

SCT—Sickle Cell Trait
SCA—Sickle Cell Anemia
SCTh—Sickle Cell Thalassemia Disease

the current was then reapplied to the other two papers for an additional 3½ hours.

For quantitative evaluation of serum protein components the usual densitometric analyses of the stained paper strips are employed. For evaluation of hemoglobin samples of about 10 per cent or more, staining is unnecessary. Trailing of proteins on the

paper during migration may introduce errors in quantitative estimations, particularly in the case of hemoglobin, in which the estimations usually involve only two constituents. In densitometric analysis of hemoglobin patterns, containing known amounts of various types, large errors were noted. The loss by trailing is particularly marked in the case of hemoglobin A, the leading type with the longest migration path, and is of importance because it can confuse the diagnosis of sickle cell thalassemia disease (microdrepanocytic anemia) (6, 7) with that of sickle cell anemia. These two conditions may be indistinguishable in terms of S and F hemoglobin values (8, 9), but the diagnosis of sickle cell thalassemia disease becomes established upon the demonstration of a small amount of A hemoglobin. Recently, cases of this condition completely lacking in hemoglobin A have been reported (10, 11). To minimize trailing, Whatman No. 1 or No. 4 papers are preferred, as are relatively low temperatures and current.

Summary. Methods are described to facilitate large scale filter paper electrophoresis. These methods permit the preparation and analysis of about 50 hemoglobin samples in a day, or the electrophoresis of large numbers of serum samples either alone or simultaneously with hemoglobin.

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EFFECT OF THIOURACIL AND METHIMAZOLE ON THYROID OF THE DOG

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The administration of thiouracil to dogs results in both hypercholesterolemia and hyperplasia of the thyroid (Steiner and Kendall, 1946; Glock, 1949). The elevated serum cholesterol returns, however, to normal within a week after thiouracil is discontinued; and the basal metabolism is depressed only fifteen to twenty per cent even when the dosage of thiouracil is increased to the limit of tolerance (Kendall, 1951). A specific effect of thiouracil on the serum cholesterol of dogs, independent of the antithyroid action, has therefore been suggested (Dock, 1951). We have found that 400 mg. of methimazole† in one oral daily dose for four weeks did not cause hypercholesterolemia in dogs (Tsai *et al.*, 1953). This observation, if interpreted uncritically, might support the possibility of a specific effect of thiouracil on serum cholesterol of dogs. This seems improbable, inasmuch as there are close similarities in the structure of both agents and their pharmacological activity is believed to be similar (Hallam and Bondy, 1951).

To study further the effect of thiouracil and methimazole on the dog's thyroid, observations were made in this experiment of serum cholesterol, serum protein bound iodine (PBI), radioactive iodine uptake by the thyroid and histological changes of the thyroid before and after administration of these two drugs. The determination of PBI and radioactive iodine uptake by the thyroid have not been utilized in the earlier studies on thiouracil in dogs; and information on the action of methimazole in dogs has been lacking.

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† With the technical assistance of A. Bennett, M. Kobayashi, M. Finley and J. R. Bateman.

Material and Methods

Eighteen healthy mongrel dogs were divided into three groups. Group I (Dogs No. 1–6) received no medication. Group II (Dogs No. 7–12) were given 1.5 gm. of thiouracil daily (Steiner and Kendall, 1946). Comparable doses of methimazole and thiouracil have not been established for dogs. For this reason, Group III (Dogs No. 13–18) were arbitrarily given 200 mg. of methimazole daily, divided into five doses in meat balls at 7 A.M., 10 A.M., 2 P.M., 6 P.M., and 10 P.M. This multiple dosage for methimazole was used, because it appears to be rapidly metabolized in the body (Hines, 1953) and one single daily dose failed to elicit hypercholesterolemia in dogs, as already stated. All dogs were observed for a period of two to four weeks. The serum cholesterol (Sperry, 1945) and PBI (Barker, 1948) were determined in each dog at the beginning and end of the experiment. Control histological sections of the thyroid were prepared in each dog by biopsy of an estimated one-fifth of the left thyroid (Markowitz, 1949) under sodium pentobarbital anaesthesia. At the end of the study, the dogs were sacrificed with intravenous sodium pentobarbital and the remaining thyroids were removed for comparative histological examination. Radioactive iodine uptake by the thyroid was measured by a tracer dose of 25 microcuries of I^{131} given intravenously 24 hours before the dogs were sacrificed. The removed thyroids were immediately subjected to direct counting. The weighed glands were placed in small planchets 10 millimeters from a scintillation counter in a lead sample changer. The counter was calibrated by the usual method with one microcurie of I^{131}. The I^{131} uptake, expressed as percentage of the tracer dose taken up by one gm. of thyroid tissue, was calculated for each dog. For serum cholesterol, PBI and thyroid histology, each dog served as its own control in addition to the comparison made among the three groups. For I^{131} uptake by the thyroid, which was determined once in each dog, Group I (no medication) served as control for Group II (thiouracil) and Group III (methimazole).

Observations

The pertinent data are recorded in Tables 1, 2, and 3. In Group I (no medication) (Table 1), the serum cholesterol

TABLE 1

Group I (No Medication)

No.	Sex-Wt. Kg.	Period of Observation (Weeks)	Serum Cholesterol Mg.% Free	Ester	Total	PBI Gammas %	Thyroid Wt. (Gm.)*	Thyroid I^{131} Uptake %/gm.	Thyroid Histology
1	M–11.5	0	27	75	102	1.1			N
		2	31	99	130	2.3	1.33	8.8	N
2	M–10.1	0	43	98	141	3.0			N
		2	25	67	92	2.6	0.68	16.9	N
3	M–11.2	0	16	68	84	1.6			N
		2	22	80	102	1.7	1.35	11.0	N
4	F–9.5	0	26	84	110	1.8			N
		3	23	66	89	3.8	0.88	20.7	N
5	M–8.3	0	18	54	72	2.3			N
		4	27	86	113	2.1	0.74	39.6	N
6	M–11.3	0	27	80	107	1.9			N
		4	37	93	130	2.3	0.98	20.2	N

* The thyroid of the dog consists of two glands with no isthmus joining them. The weight here represents the combined weight of the two glands minus the portion removed at biopsy.
N-Normal thyroid histology.
H-Diffuse hyperplasia of the thyroid.
Thyroid I^{131} uptake, %/gm. signifies the I^{131} uptake per gm. of thyroid in % of tracer dose.

TABLE 2

Group II (Thiouracil)

No.	Sex-Wt. Kg.	Period of Observation (Weeks)	Serum Cholesterol Mg.% Free	Ester	Total	PBI Gammas %	Thyroid Wt. (Gm.)*	Thyroid I^{131} Uptake %/gm.	Thyroid Histology
7	M–9.7	0	17	48	65	2.7			N
		2	60	139	199	2.0	0.72	1.5	N
8	M–10.0	0	18	67	85	2.0			N
		3	65	148	213	2.3	0.86	0.5	N
9	M–13.3	0	25	77	102	2.0			N
		3	49	125	174	3.4	1.36	0.5	N
10	M–9.7	0	45	132	179	2.0			N
		4	77	165	242	3.8	0.70	2.6	N
11	M–11.0	0	27	74	101	1.8			N
		4	140	298	438	1.5	1.30	3.0	H
12	M–7.0	0	29	99	128	1.8			N
		4	100	256	356	1.4	0.89	3.6	H

showed the same fluctuation as previously noted in a feeding experiment (Tsai *et al.*, 1954). The PBI value varied from 1.0 to 3.8 gammas per cent and fluctuated within a low range during the period of observation. The I^{131} uptake per gm. of thyroid tissue varied from 8.8 to 39.6 per cent of the tracer dose. The thyroids were histologically normal at beginning and end of the observation period.

In Group II (thiouracil) (Table 2), the I^{131} uptake per gm. of thyroid ranged from 0.5 to 3.6 per cent of the tracer dose. This represented a significantly reduced uptake as compared with the results in Group I (no medication). Diffuse hyperplasia of the thyroid was noted in two of the six dogs (Dogs No. 11 and 12) (Figs. A and B). The histological changes in the thyroid of these two dogs included irregularly shaped acini lined by tall columnar epithelium with frequent papillary infolding and scant to virtually absent colloid. There was no increase of the lymphoid tissue in the thyroids. The serum cholesterol increased in all dogs. Four of the six dogs (Dogs No. 7, 8, 11 and 12) showed an increase in cholesterol of more than 100 per cent in both the total cholesterol and the free fraction. The PBI in this group showed the same low values and variation as those in Group I (no medication). It was not affected by thiouracil administration.

In Group III (methimazole) (Table 3), the I^{131} uptake per gm. of thyroid ranged from 0.5 to 2.2 per cent of the tracer dose. This reduced uptake was comparable to the decrease in Group II (thiouracil). Five of the six dogs (Dogs No. 13, 15, 16, 17 and 18) exhibited diffuse hyperplasia of the thyroid (Figs. C and D). The histological changes of the thyroids of these five dogs were the same as those observed in Dogs 11 and 12 of Group II (thiouracil). The serum cholesterol of all the dogs in this group also showed a tendency to rise. Three of the six dogs (Dogs No. 13, 15, and 17) had an increase of more than 100 per cent in the total cholesterol and the free fraction. The PBI in this group was low as in the other two groups. It was not affected by methimazole.

Discussion

The above data indicate that both thiouracil and methimazole have a definite antithyroid effect in dogs. The most consistent

TABLE 3
Group III (Methimazole)

No.	Sex-Wt. Kg.	Period of Observation (Weeks)	Serum Cholesterol Mg.% Free	Ester	Total	PBI Gammas %	Thyroid Wt. (Gm.)*	Thyroid I^{131} Uptake %/gm.	Thyroid Histology
13	M–13.3	0	36	105	141	1.3			N
		3	136	202	338	2.0	0.99	1.5	H
14	M–13.2	0	31	77	108	0.9			N
		3	46	91	137	1.0	1.52	2.1	N
15	M–8.6	0	33	125	158	3.4			N
		3	117	215	332	1.3	0.81	1.5	H
16	M–9.5	0	48	132	182	2.0			N
		4	82	167	249	1.4	1.65	0.5	H
17	M–14.0	0	44	109	153	3.2			N
		4	99	182	281	1.4	1.68	2.2	H
18	M–14.0	0	31	49	80	3.0			N
		4	49	91	140	1.0	1.80	1.9	H
19	M–10.0	0	32	95	127	0.8			N
		2	34	79	113	1.4	0.81	4.7	N
20	M–10.0	0	36	90	126	0.6			N
		2	46	79	125	2.8	0.72	1.7	N

and sensitive method for demonstrating this antithyroid action is the measurement of the radioactive iodine uptake. The observation in two additional dogs (Dogs No. 19 and 20, Table 3) was particularly pertinent. In contrast to the multiple dosage schedule used for Group III (methimazole), Dog No. 19 received 200 mg. of methimazole in one oral daily dose for 2 weeks and Dog No 20 received the same amount in two divided daily doses. The I^{131} uptake in these two dogs was significantly depressed while the serum cholesterol and thyroid histology remained unchanged.

With the dosage and duration of thiouracil and methimazole used here, all dogs showed a depression of the I^{131} uptake. Not all of them showed, however, histological changes in the thyroid and hypercholesterolemia. The diffuse hyperplasia of the thyroid observed in Group III indicates that methimazole is a goitrogen in the dog as well as thiouracil.

Marked hypercholesterolemia occurred with greater frequency in dogs on thiouracil while diffuse thyroid hyperplasia resulted more often in dogs given methimazole. This might suggest that thiouracil has a selective effect on serum cholesterol. It

cannot be concluded from our data, however, for the variation in individual response to the drugs is great and equivalent dosage of the two drugs, in dogs, is not known. The difference in the incidence of diffuse hyperplasia of the thyroid in Group II (thiouracil) and Group III (methimazole) might be due to the single daily dose of thiouracil in contrast to the multiple daily doses of methimazole. Since both drugs cause a decrease in the radioactive iodine uptake and diffuse hyperplasia of the thyroid, and methimazole may also cause hypercholesterolemia in dogs when administered in multiple daily doses, it seems that the thiouracil induced hypercholesterolemia in dogs is an integral part of its antithyroid effect.

The PBI is lower in dogs than in men. The low PBI values observed in this experiment were not due to any fault of the method (Barker, 1948), which has been used by us on patients with accurate and reproducible results. Barker (1948) himself reported a low value of 2.3 gamma per cent in a series of eight dogs. Since low PBI may be due to hypoproteinemia as in nephrotic patients (Peters and Man, 1948), determinations of serum protein (Kingsley, 1942) and PBI were made on the same specimens of blood in another five healthy mongrel dogs (Dogs No. 21–25) (Table 4). Serum proteins in these dogs were slightly lower than the

TABLE 4

Serum PBI and Proteins on Identical Blood Samples in Dogs

No.	PBI Gammas %	Total Serum Protein gm. %	Albumin gm. %	Globulin gm.%
21	2.4	5.22	3.08	2.14
22	2.3	5.91	4.44	1.47
23	2.4	5.77	3.52	2.25
24	2.6	5.80	3.68	2.12
25	1.9	5.62	3.58	2.04

normal range in men, but do not appear to be low enough to account for the low PBI values. Our data indicate that the normally low PBI in dogs is not reduced by thiouracil or methimazole, even though the thyroid hyperplasia due to the goitrogenic effect of the two drugs can be best explained on the assumption that the circulating thyroid hormone has decreased. This suggests that the circulating thyroid hormone in dogs may not be totally bound to the serum protein. It is concluded that the deter-

mination of PBI, like basal metabolism determination (Glock, 1949), is not a valid test of decreased thyroid function in dogs.

Summary

Both thiouracil and methimazole exert an antithyroid action in the dog. A decreased radioactive iodine uptake by the thyroid is the most sensitive method of demonstrating this action. Diffuse hyperplasia of the thyroid that may follow the administration of the two drugs indicates that they are goitrogens in dogs as well as in other mammals. Since methimazole may also induce hypercholesterolemia in dogs, this effect of thiouracil in dogs is mediated at least partly through its antithyroid action. The serum protein bound iodine is normally low in dogs and is not influenced by either thiouracil or methimazole.

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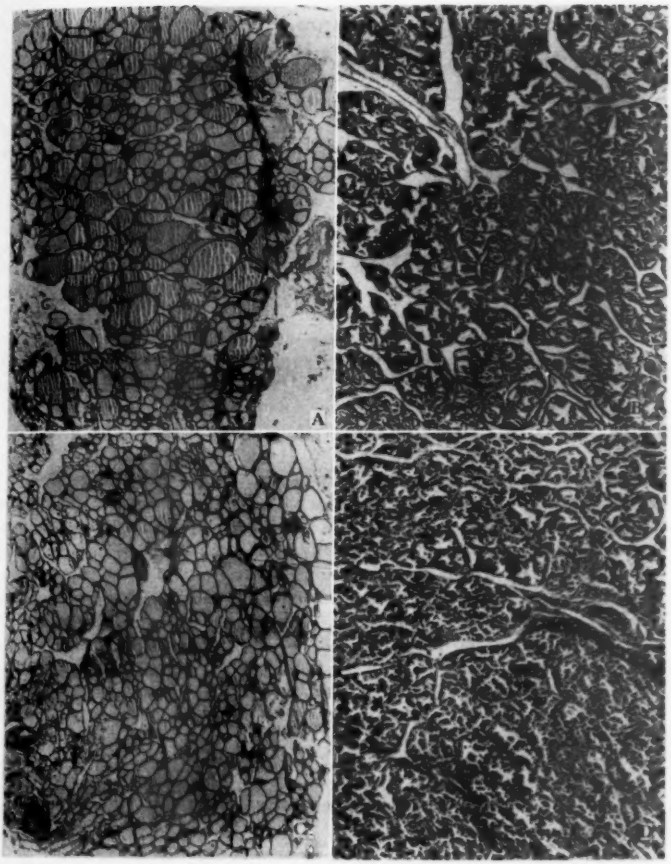

PLATE I

FIG. A: Shows normal thyroid architecture of biopsy specimen on Dog #11.

FIG. B: Shows the hyperplastic reaction following thiouracil administration in Dog #11.

FIG. C: Shows normal thyroid architecture of biopsy specimen on Dog # 15.

FIG. D: Shows hyperplastic reaction following methimazole administration in Dog #15.

All stained in Hematoxylin and phloxine, and magnified 42x.

CALLING ATTENTION TO

Items of Possible Interest to Our Friends

May, 1956

General: GM Clemence discusses new standards of time and frequency (*Science* 123/567/Apr 6/56). J. B. Conant assaults *The Citadel of Learning* (Yale Univ Press, New Haven, Conn., 56, 86pp, $2). P Diepgen issues *Geschichte der Medizin: Die Historische Entwicklung der Heilkunde und des arztlichen Lebens* (Springer, Berlin, 49–56, 3 vols, 950pp, with Bilderatlas, $35). I Galdston edits *Ministry & Medicine in Human Relations* (Internat Univ Press, NY, 56, 173pp, $3.5). RM Lambert introduces memorial tributes to AM Pappenheimer 1878–1955 (*Lab Invest* 5/1–131/56). J Lear edits new section of *Sat Rev* on Science & Humanity, & many predictions are made (*Sat Rev* Mch 24/56). S Mandel gives story behind "The Search for Bridey Murphy" (*Sat Rev* Mch 10/56). RA McIver edits *Great Moral Dilemmas* (Harper, NY, 56, 230pp, $3). HT & RT Northern give *The Secret of the Green Thumb* (Ronald Press, NY, 56, 431pp, $5). J Perry offers *The Story of Standards* (Funk & Wagnalls, NY, 56, 271pp, $5). AV Neale edits Thomas Phaire's 1545 *Boke of Children*, 1st English text on pediatrics (Livingstone, Edinburgh, 55, 76pp, 7s6d). B Rouerche embellishes annals of medicine with keen account of aspirin (*The New Yorker*, Mch 31/56, p39ff). E Wilson examines Zunis, Haitians, Russians & Israelis in *Red, Black, Blond & Olive* (Oxford Univ Press, NY, 56, 278pp, $6.75).

Clinical: Our JMB Bloodworth & E Von Haam find no correlation between catechol amine concentration in heart and disease (*Circul* 13/573/56). E Durbin & LJ Goldwater discuss rehabilitation of cardiac patients (*Circulation* 13/410/56). FG Germutti Jr surveys adrenocortical steroids in infection & immunity (*Pharmacol Rev* 8/1/56). RJ Jones finds reduction of serum cholesterol after 30 gms daily oral beef brain extract free from protein (*J Lab Clin Invest* 47/261/56). JH Lawrence & CA Tobias are conservative in reviewing cancer therapy with radioactive isotopes & nuclear radiation (*Cancer Res* 16/185/56). CA Macgregor & RR Pfister note value of polyethylene glycol ointment in exposure treated burns (*Surgery* 39/557/56). IA Mirsky, WL Miller, WE Dulin, LW Kinsell & Co report on oral sulfanylureas ("orinase") as insulin sparing agents (*Science* 123/583, 584, 585, Apr 6/56). LC Reid, AB Clark & HA Rusk review protein metabolism in injury and convalescence (*Postgrad Med* 19/206/56). F Stohlman Jr & G Brecher find anemic plasma factor as erythropoietic stimulant in sublethal irradiation injury (*Proc Soc Exp Biol Med* 91/1-56). JH Talbott & RM Ferrandis review *Collagen Diseases* (Grime & Stratton, NY, 56, 256pp, $6.5). SC Werner edits *The Thyroid*, a compilation by 60 contributors (Hoeber, NY, 56, 800pp, $20). *Federated Societies for Experimental Biology* breaks all records: 2034 abstracts by over a 1000 workers from LD Abbott to RE Zwicky (*Fed Proc* 15/1–624/56).

Preclinical: TS Argyris finds thiamine deficiency brings atrophy of sebaceous glands in skin (*Science* 123/634/Apr 13/56). E Braun-Menendez

discusses pharmacology of renin (*Pharmacol Rev* 8/25/56). BG Brown & P Hey find choline phenyl ethers are potent amine oxidase inhibitors (*Brit J Pharmacol* 11/58/56). Our NM Buckley, M Sidky & E Ogden study effects of epinephrine on ventricular filling (*Circul Res* 4/148/56). MB Chenoweth reviews chelation as pharmacological mechanism (*Pharmacol Rev* 8/57/56). G Falk notes adenosine triphosphate muscle contracting action is due to calcium binding (*Science* 123/632/Apr 13/56). JH Gaddum & NJ Giarman study biosynthesis of 5-hydroxytryptamine (*Brit J Pharmacol* 11/88/56). HS Gasser finds olefactory nerve fibers conform to general properties of vertebrate nerves (*J Gen Physiol* 39/473/56). FE Hunter Jr & DH Lowry review drug action on enzymes (*Pharmacol Rev*, 8/89/56). A Kasanen & I Viitanen find increased copper in thyroid in any kind of goiter (*Acta Med Scand* 153/467/56). L Matko & Co find karyclastic substances as colchicine & nitrogen mustard act on cell nuclei both in dividing & resting cells, inhibiting mitosis with nucleolar injury (*Acta Morph Acid Sci Hungaricae* 6/299/56). WD Russell & Co study cancer eye in cattle (*Cancer* 9/1/56). AW Wase & YSL Feng show salivary glands influence thyroid function (*Nature* 177/624/Mch 31/56).

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MEDICAL: ZM Bacq & P Alexander consider *Principles des Radiobiologie* (Masson, Paris, 1956, 478pp, 3600 Fr). JW Brasch & FH Ellis give evidence of a gastroesophageal sphincter mechanism (*Surg* 39/901/56). BA Brodie & Co show that only rauwolfia alkaloids which cause sedation can affect brain serotonin concentration (*Science* 123/992/June 1/56). LS Fosdick finds N-lauroyl sarcosinate in dentifrices reduces dental caries activity (*Science* 123/988/June 1/56). NS Kline edits *Psychopharmacology Symposium* chiefly on chlorpromazine & reserpine (American Asso. Advancement Science, Washington DC, 56, 175pp, $3.5). A & B Pullman well discuss *Cancerisation par les Substances Chimiques et Structure Moléculaire* (Masson Paris, 55, 306pp, 2800 Fr). FB Rea discusses *Alcoholism: Its Psychology & Cure* (Philosophical Library, NY, 56, 143pp, $3.5). Science Council of Japan compiles results of *Research on the Effects & Influences of the Experimental Hydrogen & Atomic Bomb Explosions*, including 48 medical articles (Nippon Gakujutsu Shinkokai Tokio, 56, 2 vols, 1700pp, $26.5). SR Slavson plows *The Fields of Group Psychotherapy* (Internat Universities Press, N.Y., 56, 338pp, $6). WW Westerfield well analyzes drug-effect relations (*Science* 123/1017/June 8/56). V du Vigneaud follows the trail of sulfur research from insulin to oxytocin (*Science* 123/967/June 1/56). Our RM Zollinger & RD Wiliams consider surgical aspects of jaundice (*Surg* 39/1016/56).

GENERAL: L & RR Ansbacher analyse *The Individual Psychology of Alfred Adler* (Basic Books, NY, 56, 503pp, $7.5). FC Barghoom skillfully wades through the maze of *Soviet Russian Nationalism* (Oxford Univ Press, NY, 56, 330pp, $7). Don Blanding comes back with *Joy Is An Inside Job* (Dodd Mead, NY, 56, 160pp, $2.75). Z Chafee Jr is optimistic about *The Blessings of Freedom* (Lippincott, Phila., 56, 350pp, $5). WO Douglas goes on an interesting *Russian Journey* (Doubleday, NY, 56, 255pp, $4.5). JF Fulton

gives worthy appreciation of John P. Peters of Yale, who was hounded by the authoritarians (*Science* 123/1023/June 8/56). A. Gesell & Co tackle *Youth: Years from Ten to Sixteen*, and find it keen (Harper, NY, 56, 422pp, $5). A Gregg makes pertinent *Challenges to Contemporary Medicine* (Columbia Univ Press, NY, 56, 96pp, $2). L & E Hanson review *The Tragic Life of Toulouse-Lautrec* (Random House, NY, 56, 277pp, $5). E Hitchman dissects *Great Men: Psychoanalytic Studies* (International University Presses, NY, 56, 278pp, $4). MV Kline edits *A Scientific Report on "The Search for Bridey Murphy"* with much sense, now the solution is clear (Julien Press, NY, 56, 224pp, $3.5). Gourmet & bon vivant chef G Mardikian sings *Song of America* (McGraw-Hill, NY, 56, 320pp, $4.5). Architect R Neutra philosophizes on *Life and Human Habitat* (A Koch, Stuttgart, 56, 318pp, $18). SF Olson enjoys *The Singing Wilderness* (AA Knopf, 56, NY, 245pp, $4). LJ Saul deplores *The Hostile Mind* (Random House, 56, NY, 260pp, $3.5). SM Shane popularizes *Anesthesia: Thief of Pain* (Vantage Press, NY, 56, 87pp, $2.5). DA Stewart offers *Preface to Empathy* (Philosophical Library, NY, 56, 157pp, $3.75). R Suter opens *A Gallery of Scientists* (Viking, NY, 56, 132pp, $3). FX Sutton & Co look into *The American Business Creed* (Harvard Univ Press, Cambridge, Mass, 56, 416pp, $6.75). O von Sunson studies *The Gothic Cathedral: Origin of Gothic Architecture & the Mediaeval Concept of Order* (Pantheon, NY, 56, 307pp, $6.5). W Winslow tells excitedly *The Menninger Story*, giving all the dope on Doctor Karl and Doctor Bill (Doubleday, NY, 56, 350pp, $5).

NOTE: This is being written from Princeton under the stress of trying to get an exhibit completed on *Some Founders of Physiology* for the XXth International Congress of Physiology to be held in Brussels July 28–August 4, 1956. This has resulted in a souvenir book, which is far from satisfactory, and which, it is hoped, may be more adequately prepared for later distribution. It is not likely that there will be *Calling Attention To* for August, and whether it can be continued subsequently is doubtful. Here's best greetings!

CHAUNCEY D. LEAKE